Conservation management to protect the threatened ptunarra brown butterfly (*Oreixenica ptunarra*) from the threat of predation by introduced vespid wasps in Tasmania, Australia

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Declaration

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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Josephine Potter-Craven

15 May 2019

Statement of Co-Authorship

The following people and institutions contributed to the publication of work undertaken as part of this thesis:

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Abstract

Butterfly numbers are declining worldwide primarily due to habitat loss and habitat degradation, resulting in many species now being classified as threatened. Many butterfly species are further endangered by the additional threats of fragmentation, agricultural chemicals, climate change and introduced predators. In Tasmania, Australia, the threatened ptunarra brown butterfly (*Oreixenica ptunarra*) has recently come under threat from predation by introduced vespid wasps, which have the potential to further reduce their numbers, possibly causing local extinctions. The nature of this new threat and possible conservation actions to mitigate it are the subject of this thesis.

The present study investigated whether vespid wasps were having a significant impact on *O. ptunarra* numbers, ways of controlling the wasps or excluding them from *O. ptunarra*'s habitat, and methods of creating new populations of *O. ptunarra* by translocating individuals within its historical range. Vespid wasp control was performed by using toxic baits containing fipronil, as well as directly poisoning wasp nests. Transect surveys to count vespid wasp and *O. ptunarra* numbers performed at wasp control sites and monitoring sites, were compared to determine whether the wasp control was effective and whether the wasps were having a negative effect on *O. ptunarra* numbers. Vegetation transect surveys were also performed at sites with and without *O. ptunarra* to determine the relationship between flora species and *O. ptunarra* numbers and which species *O. ptunarra* prefers. Translocations of *O. ptunarra* were attempted by moving female imagoes and eggs to suitable sites within the species' historical range to create new, self-sustaining populations. The buffer size necessary to exclude wasps from *O. ptunarra* habitat was also investigated by analysing the vegetation around the *Poa* grasslands that comprise *O. ptunarra*'s habitat at various buffer distances, using GIS. The vegetation analysis was compared to vespid wasp and *O. ptunarra* numbers to determine which vegetation types affected their numbers and the buffer size required to exclude wasps from *O. ptunarra* habitat.

It was determined that vespid wasps were having a negative impact on *O. ptunarra* numbers and that, when wasp numbers were controlled, *O. ptunarra* numbers rose marginally. Unfortunately, wasp numbers were not decreased enough to protect *O. ptunarra* sufficiently, suggesting that wasp control efforts need to be increased. *O. ptunarra* was successfully translocated to one of four release sites, with a persistent population of butterflies being detected in the following four years. Analysis of the vegetation composition at the sites determined that *O. ptunarra* preferred sites containing the species *Poa labillardierei* and *P. hiemata* and a high abundance of flowering nectar plant species. These attributes should be taken into consideration during the selection of potential sites for future translocations of *O. ptunarra.* The buffer analysis determined that plantation forests were having a significant negative effect on *O. ptunarra* numbers and a positive effect on vespid wasp numbers. A buffer around the *Poa* grasslands of 300-500 m, containing native vegetation and excluding plantation forest, was suggested as a means to exclude vespid wasps and further protect *O. ptunarra*.

Table of Contents

Declaration ii
Statement of Co-Authorship..... iii
Acknowledgements iv
Abstract v
Table of Contents vi
Chapter 1 Introduction 1
1.1. Butterfly conservation 1
1.1.1. Habitat loss 1
1.1.2. Fragmentation and edge effects 2
1.1.3. Habitat degradation..... 2
1.1.4. Climate change 2
1.1.5. Agricultural chemicals & GM 3
1.1.6. Introduced predators 4
1.2. Conservation actions 4
1.3. Research gaps 6
1.4. Aim and objectives 7
Chapter 2 Oreixenica ptunarra review 8
2.1 Description..... 8
2.2 Lifecycle 9
2.3 Distribution and habitat 11
2.4 History of conservation and recovery planning for *O. ptunarra* 12
Chapter 3 The effects of introduced vespid wasps (Vespula germanica and V. vulgaris) on threatened native butterfly (Oreixenica ptunarra) populations in Tasmania 17
Abstract 17
3.1 Introduction..... 17
3.2 Methods..... 19
3.2.1 Study sites..... 19
3.2.2 Data collection..... 20
3.2.3 Wasp control 22
3.2.4 Data analysis..... 24
3.3 Results 25
3.4 Discussion 29

3.5 Conclusion 31

Chapter 4 A successful translocation of the threatened ptunarra brown butterfly (Oreixenica ptunarra) in the highland Poa grasslands of Tasmania, Australia 32

Abstract 32

4.1 Introduction 32

4.2 Methods..35

4.2.1 Translocation 35

4.2.2 Monitoring 39

4.2.3 Wasp control 40

4.2.4 Vegetation surveys 40

4.2.5 Data analysis 40

4.3 Results 41

4.3.1 Translocation 41

4.3.2 Wasp control 41

4.3.3 Vegetation surveys 42

4.4 Discussion 43

4.5 Conclusion 46

Chapter 5 Optimal width of buffer zones around Poa grasslands to protect threatened Oreixenica ptunarra butterflies from predatory vespid wasps 47

Abstract 47

5.1 Introduction 47

5.2 Methods..49

5.3 Results 51

5.3.1 Vegetation types - overall..51

5.3.2 Vegetation types – by site 52

5.3.3 Correlations 55

5.3.4 Example site – Peak Plain 57

5.4 Discussion 59

5.5 Conclusion 61

Chapter 6 Discussion 62

6.1 The impacts of vespid wasps on *O. ptunarra* 62

6.2 Management of vespid wasps for the conservation of *O. ptunarra* 63

6.3 Further conservation management..65

6.3.1 Recovery planning 65

6.3.2 Reducing habitat loss 66
6.3.3 Habitat management 66
6.3.4 Agricultural chemicals & GM 67
6.3.5 Climate change 67
6.3.6 Captive propagation 68
6.4 Conclusion 68
Chapter 7 References 69
Appendix A 78

Chapter 1 Introduction

1.1. Butterfly conservation

Butterfly numbers are declining worldwide primarily due to habitat loss and habitat degradation, resulting in many species now being deemed at-risk (Schultz et al. 2008; van Strien et al. 2019). Further threats to butterflies include fragmentation of habitat, climate change, the use of pesticides, herbicides, fertilisers, GM crops, and introduced predators. In the Netherlands, an 84% decline in butterfly numbers since 1890 has been detected (van Strien et al. 2019). In the UK 71% of butterfly species have declined over approximately 20 years, with other countries such as Belgium, Finland and the USA also showing declines in butterflies (Breed et al. 2012; Thomas et al. 2004; van Strien et al. 2019).

Butterflies are similarly in decline in Australia, with six butterfly species now listed as threatened under federal environmental legislation and several more under State legislation (Department of Environment and Energy 2019). Within Tasmania, there are five threatened butterflies, the Tasmanian chaostola skipper (*Antipodia chaostola leucophaea* Couchman, 1946), Marrawah skipper (*Oreisplanus munionga larana* Couchman, 1962), Tasmanian hairstreak butterfly (*Pseudalmenus chlorinda myrsilus* Westwood, 1851), chequered blue butterfly (*Theclinesthes serpentata lavara* Couchman, 1954), and ptunarra brown butterfly (*Oreixenica ptunarra* Couchman, 1953).

The conservation management of the threatened ptunarra brown butterfly (*O. ptunarra*), a species endemic to the *Poa* grasslands of Tasmania, is the focus of this thesis. The term 'conservation management' is used to refer to the management of the species for conservation purposes, in order to reduce negative impacts upon it and to assist in its ongoing survival. Negative impacts may be reduced by a series of 'conservation actions'.

The main threat to *O. ptunarra* is habitat loss, with other threats including fragmentation of habitat, inappropriate fire and grazing regimes (Threatened Species Unit 1998), the overspray of pesticides and herbicides unintentionally impacting on the butterflies (Neyland and Brown 1996), changing landscapes due to climate change, and predation by vespid wasps. More detailed information on the description, lifecycle, distribution and conservation history of *O. ptunarra* is provided in Chapter 2.

To restore threatened butterfly populations a combination of conservation actions can be undertaken, including: habitat restoration, translocation, captive propagation (Schultz et al. 2008) and addressing threatening processes. The various threats to butterflies and the conservation actions that can be used to mitigate them are outlined below.

1.1.1. Habitat loss

Habitat loss due to clearing of native vegetation for land uses such as forestry plantations, agriculture and urban expansion is occurring globally and has resulted in range contractions and species extinctions over the last two centuries (Newbold et al. 2016; Pimm et al. 2014). Habitat loss is also considered to be one of the main drivers of biodiversity decline worldwide (Haddad et al. 2015). Habitat loss has reduced butterfly numbers globally (Schultz et al. 2008; Thomas et al. 2004; van Swaay 1990) and has been identified as the greatest threat to butterflies in Australia, as the butterflies' environment and host plants are removed (Sands and New 2002).

1.1.2. Fragmentation and edge effects

A consequence of habitat loss is that the remaining habitat is often fragmented into smaller remnant patches (Herse et al. 2018). These habitat fragments are frequently surrounded by the converted landscapes with their intensified land uses. Negative edge effects often occur along the border between the natural fragment and the adjacent converted habitat, further compounding the negative effects of the other changes to the ecosystem (Frost et al. 2015).

Examples of negative edge effects include increased parasitism, interspecific competition and predation risk, and abiotic factors such as changes in temperature (Herse et al. 2018; Pérez-Rodríguez et al. 2018; Schneider et al. 2013). Edge effects are often due to a spillover of dispersing or foraging organisms across habitat borders (Frost et al. 2015). For example, predators spilling over from coniferous forests into adjacent calcareous grasslands in Germany lead to higher predation rates on the grassland fragments (Schneider et al. 2013). Negative edge effects can result in a further decline in biodiversity in the habitat fragment and are often strongest at the edges of neighbouring habitats (Schneider et al. 2013), but can range further, with effects on beetle communities extending up to 1 km into forests in New Zealand (Ewers and Didham 2008). The spillover of introduced predatory vespid wasps is a risk to native invertebrate species, including lepidopterans, which have been shown to be negatively affected by vespid wasp predation (Beggs and Rees 1999).

1.1.3. Habitat degradation

The unsuitable management of butterfly habitat often leads to habitat degradation, resulting in a decline in butterfly numbers or local extinctions. The natural or semi-natural disturbance regimes required to maintain butterfly habitats have been widely disrupted by human activities (Schultz et al. 2008). Butterfly species in temperate areas that occupy open vegetation types at an early successional stage such as grasslands, moorlands and prairies, require their sites to have an open structure so that the butterflies receive enough sunlight for warmth to enable flight, and so that the vegetation is open enough for effective ovipositing and for a variety of flowering herbs to grow for nectaring (Smallidge and Leopold 1997). Methods of maintaining the open, early successional structure required for butterflies generally involve various ways of removing woody shrubs, so that the host plants do not become overgrown, and reducing grassland biomass to create the space required within the vegetation for flowering plants to grow. Ways of doing this include patch burning, grazing, and manual vegetation removal, such as coppicing or complete removal of woody vegetation and weeds. Haying and mowing can also be used to reduce biomass in grasslands but are not practised in the highland *Poa* grasslands of Tasmania.

Inappropriate grazing and fire regimes can have a negative impact on butterflies such as *O. ptunarra* (Neyland 1993). Fire and grazing regimes need to occur at a suitable frequency and season, otherwise they risk unintentionally decreasing the habitat suitability, resulting in a reduction in butterfly numbers instead of an increase.

1.1.4. Climate change

Human induced climate change has caused global temperatures to rise over the last 150 years, especially over the last six decades. This warming has triggered other changes to the Earth's climate such as melting glaciers, rising sea levels, changes in rainfall patterns and storms (Wuebbles et al. 2017). Consistent with this trend, Australia has warmed, rainfall patterns have changed, sea surface temperatures have increased, and sea levels have risen (Hughes 2003).

Climate, particularly temperature, affects the distribution of butterflies, mainly through changes in the vegetation composition of their habitats (Beaumont and Hughes 2002). Changes in temperature can also cause morphological changes in the butterflies (Beaumont and Hughes 2002). As the climate warms, butterflies' ranges are predicted to shift polewards or to higher altitudes, with the extinction of lower latitude and elevation populations (Parmesan et al. 1999). It is also predicted that butterflies will emerge earlier and have longer flight periods (Beaumont and Hughes 2002; Roy and Sparks 2000). British butterflies have been shown to respond to warmer, drier conditions in many ways, including changes in voltanism, dates of emergence, delayed maturation of eggs (Pollard and Yates 1993), higher survival rates and improved oviposition (Roy 2001). Some butterfly habitats have already undergone changes, but the movement of butterfly populations to new habitats has been slower than expected, often with a time lag (Menéndez et al. 2006). Most of the effects of climate change on butterflies are predicted to be positive, as the increased temperatures will allow the butterflies to spend less time sheltering and spend more time flying, nectaring, laying eggs and dispersing (Roy and Sparks 2000). The main negative effects are predicted to be associated with drought affecting host plant growth, habitat composition and structure and egg survival (Roy and Sparks 2000).

A large group of Australian endemic butterflies were modelled to predict their response to climate change, with 88-92% of species showing a decrease in their ranges under various climate change scenarios, with only 22-63% of the future ranges overlapping with the current ranges (Beaumont and Hughes 2002). This has implications for future management of these species as their ranges shift. A subset of butterfly species were more closely studied, with *Oreixenica correae* Olliff, 1890 (correa brown butterfly), the most closely related taxon to *O. ptunarra,* showing an 18.9% decrease in its range under the most extreme climate change scenario, with less than 30% of the future range overlapping with the current range (Beaumont and Hughes 2002). This suggests that *O. ptunarra* should also be monitored for potential climate induced changes, which will potentially change the landscape in which *O. ptunarra* resides.

Climatic changes that Tasmania has already experienced include increased rainfall variability, resulting in extreme dry and wet periods, and an increased occurrence of dry lightning which is promoting more wildfires (Styger et al. 2018). Climate change modelling additionally suggests that Tasmania will get drier and warmer in the highlands (Grose et al. 2010). These changing conditions are likely to alter the distribution of vegetation communities, which will presumably follow their climatic niche, either moving to higher altitudes or south to cooler areas that now have the required ecological conditions (Hughes 2003). As highland *Poa* grasslands, *O. ptunarra*'s habitat, occur at higher elevations, it is likely that they will be affected by climate change.

1.1.5. Agricultural chemicals & GM

Agricultural practices such as the spraying of pesticides and herbicides, application of fertilisers, and the use of genetically modified (GM) crops also pose a threat to butterflies. This threat has increased as many western countries have shifted to large-scale monoculture crops that required large doses of chemicals or GM treatments to reduce damage by pests and maintain profits (Altieri 2000; Robinson and Cowling 1996). Butterflies can be exposed to pesticides and herbicides through direct overspraying or inadvertent spray drift or vapour drift, with aerial spraying often resulting in greater non-target exposure than using ground-based equipment (Longley and Sotherton 1997). Pesticide use can kill all butterfly life stages outright, while sublethal doses may impede their growth (Longley and Sotherton 1997). For example, when individuals of *Pieris brassicae* Linnaeus, 1758 (large white butterfly) were exposed to sublethal doses of pyrethroids they experienced extended larval times, a reduction in weight gain, and smaller adults (Tan 1981). Pesticides in the pyrethroid group are particularly toxic for butterflies, which is not surprising as they were originally designed to have a high potency against lepidopteran agricultural pests (Elliott et al. 1978). Herbicides can affect butterflies by eradicating their host plants or their flowering nectar sources, which may lead to the starvation or lowered fitness of individuals (Longley and Sotherton 1997). Fertilisers can negatively affect native habitats, as they increase the nutrient availability of the soil, which can change the composition of the flora growing at the site (Longley and Sotherton 1997).

Genetically modified crops can also negatively affect butterflies, resulting in mortality or reduced growth rates. For example, the larvae of the monarch butterfly (*Danaus plexippus* Linnaeus, 1758), when fed leaves of their host plant dusted with GM corn pollen transformed with genetic material from the bacterium *Bacillus thuringiensis* Berliner, 1915 (*Bt*), were found to feed more slowly, grow at a slower rate, attain a lesser body weight and have a significantly higher mortality rate than larvae fed on plain leaves, in the USA (Losey et al. 1999). Fortunately, due to low levels of exposure, *Bt* corn generally poses a low risk to *D. plexippus*, (Koch et al. 2003). More recently, there has been a surge in the production of genetically modified glyphosphate resistant maize and soybeans in the USA, resulting in a higher use of glyphosphate herbicide in the agricultural landscape, which has been linked to a decline in the food plant (milkweed, *Asclepias* spp. L.) of the monarch larvae (Belsky and Joshi 2018).

1.1.6. Introduced predators

Introduced species are increasingly becoming an environmental problem due to increased shipping and movement of people, and are regarded to be the second greatest threat to endangered species in the USA, behind habitat loss (Wilcove et al. 1998). Invasive species can cause damage by outcompeting or predating upon native species, which can cause a change in species composition, niche shifts and local extinctions (Eastwood et al. 2007). Social insects such as wasps and ants are amongst the most concerning introduced species, as they are able to proliferate quickly and are usually generalist predators, enabling them to rapidly have a large impact on native species. Introduced species that have been documented to have impacts on native butterflies include the multicoloured Asian lady beetle (*Harmonia axyridis* Pallas, 1773) predating the eggs of the monarch butterfly in the USA (Koch et al. 2006), introduced biocontrol agents parasitising or predating upon non-target butterfly species in Guam (Nafus 1993), and vespid wasps predating on a variety of invertebrates including lepidopterans (Beggs and Rees 1999).

Vespid wasps are an introduced pest in Argentina, South Africa, USA and New Zealand, where wasp control trials have been performed to reduce their numbers (Beggs et al. 2011; Hanna et al. 2012; Sackmann et al. 2001; van Zyl et al. 2018). Vespid wasps have had a significant negative impact on native invertebrates, such as lepidopterans, through predation in New Zealand (Beggs and Rees 1999; Harris 1991). Vespid wasps are also an introduced pest in south-eastern Australia, including the island of Tasmania (Kasper et al. 2008). Very little research has been conducted into the impact of vespid wasps on invertebrates in Australia.

1.2. Conservation actions

The restoration of threatened butterfly populations usually involves a combination of three main conservation actions: habitat restoration, translocation, and captive propagation (Schultz et al. 2008) but can also include other actions such as the reduction of threats posed by predators or chemical sprays, or the creation of buffers to protect butterfly habitat.

The majority of restoration activities are conducted to maintain open or early successional habitat for the target butterfly species. In the UK, butterfly habitat degradation is largely due to a change in land use resulting in a reduction in grazing and coppicing of sites, while in the USA it is due to the rapid spread of introduced weed species or for insect management issues (Baker and Potter 2018; James et al. 2015; Schultz et al. 2008).

Degradation can also be caused by negative edge effects in fragmented landscapes (Frost et al. 2015). Habitat restoration is often accomplished by grazing, burning or mowing the site and removing woody shrubs and weeds that would otherwise take over the site. Additional works to enhance the site are sometimes performed if necessary and include actions such as planting host plants, nectar plants, and other native plants as well as engineering the site to enhance hydrology, soil, vegetation structure and connectivity (Schultz et al. 2008). For example, the heath fritillary butterfly (*Melitaea athalia* Rottemburg, 1775) was successfully restored to rehabilitated sites in the UK that had previously become overgrown due to a reduction in the practice of coppicing. At some sites butterfly populations increased naturally, while other sites required populations to be reintroduced (Pullin 1996).

The translocation of butterflies involves moving individuals (adults, pupae, larvae, eggs, or a mixture thereof) from a source site to a new site containing suitable habitat to start a new, self-sustaining population. This usually occurs within the historical range but more commonly occurs outside the previous range, in order to populate offsets for habitat loss due to developments and other land use changes, as well as a change in habitat suitability caused by climate change (Seddon 2010). Most of the threatened butterfly recovery strategies in the USA and UK recommend translocation as a possible conservation action, the outcome being that translocations had been attempted for most threatened butterfly species in the UK and for several species in the USA by 2008 (Schultz et al. 2008). For example, the large blue butterfly (*Phengaris arion* Linnaeus, 1758) was successfully translocated in the UK, once its habitat had been restored after becoming overgrown due to changes in land use (Pullin 1996), while the silver-studded blue butterfly (*Plebejus argus* Linnaeus, 1758) was successfully translocated to nearby areas of suitable habitat in the UK (Brookes 1997). In NSW, Australia, the purple copper butterfly (*Paralucia spinifera* Edwards & Common, 1978) was successfully translocated after its habitat was accidently removed during roadworks (Mjadwesch and Nally 2008).

Captive breeding or propagation of butterfly species is an ex-situ method used for rearing large numbers of butterflies to be released into the wild either for translocations or to enhance the numbers of wild stock (Schultz et al. 2008). Adults are usually captured for use as breeding stock, while a variety of life stages are released (Schultz et al. 2008). This is usually only carried out with species that are easily propagated, within large, well-equipped institutions such as zoos and butterfly houses. While this method has promise, as a large numbers of individuals can be reared for release, there are disadvantages, such as morphological or genetic changes, disease and financial restraints (Schultz et al. 2008). For example, in the UK, captive-bred large white butterflies (*Pieris brassicae*) had smaller wings and were heavier, which affected their ability to disperse when released (Lewis and Thomas 2001), while the Palos Verdes blue butterfly (*Glaucopsyche lygdamus palosverdesensis* E. Perkins & J. Emmel, 1977) captively reared in the USA, suffered losses due to microsporidian infection (Mattoni et al. 2003).

The reduction of threats such as chemical sprays and introduced predators is also important for the conservation of butterflies. Leaving unsprayed buffer areas around butterfly habitat has been found to be effective in reducing the non-target mortalities caused by spray drift in the UK (Longley et al. 1997). Spray drift of pesticides and herbicides can also be minimised by taking into account the wind-speed and direction and by avoiding spraying during windy conditions (Nordby and Skuterud 1974). Additionally, when spraying from the ground, having a low boom height of 40 cm and a low spray pressure of 2-5 bar was found to reduce spray drift by more than half (Nordby and Skuterud 1974).

The reduction in predation impact on butterflies by introduced species is also important for their ongoing persistence. Predation impacts on butterflies are recorded occasionally but often no further control is performed to reduce predator numbers. The group for which the greatest amount of research has been performed and for which large-scale control trials have been performed are the vespid wasps, which are generalist predators that consume a variety of invertebrates including lepidopterans (Beggs 2000; Hanna et al. 2012; Harris and Etheridge 2001; Sackmann et al. 2001). Control methods include directly poisoning the nests, toxic baiting and biological control. Wasp numbers must be reduced to very low levels for the control to be effective (Beggs and Rees 1999; Toft and Rees 1998). Directly poisoning nests with an insecticide like permethrin is effective but it is often difficult to detect all the nests in a large landscape (Beggs et al. 1998). Biological control would potentially be more effective over large areas, but trials to date have proven ineffective (Beggs et al. 2008). The most common form of wasp control is toxic baiting, whereby wasps take some meat bait containing insecticide back to their nest, effectively poisoning the nest and themselves. Prevalent toxins used are 1080 (sodium fluoroacetate), sulfluramid and fipronil, with the latter currently being the most effective and commonly used poison (Beggs et al. 1998; Harris and Etheridge 2001).

1.3. Research gaps

Tasmania has five threatened butterfly species listed under State legislation, with possibly more at risk. The nature of the threats to the listed species have been assessed but generally not thoroughly investigated. The most researched of the species is *O. ptunarra* and the current study increases knowledge of the interaction between this butterfly, its environment and vespid wasps in the hope of providing better guidelines for recovery.

Predation by vespid wasps has recently been identified as a potential threat to *O. ptunarra* (Bell 1999). Wasps have been observed catching butterflies, biting off their wings and carrying the bodies back to the nest to feed their young (P. Bell pers. comm.). There are two types of vespid wasp present in Tasmania, the German wasp (*Vespula germanica* Fabricius, 1793) and the English or common wasp (*Vespula vulgaris* Linnaeus, 1758). Since their establishment in Tasmania in 1959 and 1995 respectively (Matthews et al. 2000), the wasps have been expanding their ranges throughout the State. They have only been documented in *O. ptunarra* sites in north-western Tasmania since the late 1990s (P. Bell pers. comm.). Limited research has been performed on the impact of vespid wasps on native species, or the effectiveness of wasp control, in Tasmania or elsewhere in Australia (Spradbery and Maywald 1992). The main focus for wasp research in Tasmania has been crop protection rather than conservation, as wasps can cause costly impacts to agricultural crops such as soft fruits (eg. Statham and Warren 2002). The quantum of the impact of vespid wasps on *O. ptunarra* by predation is unknown and could potentially be devastating, as further reductions in *O. ptunarra* numbers could lead to a decline in genetic variability and local extinctions. The present study investigated the impacts of vespid wasps on *O. ptunarra* numbers and trialled wasp control methods to reduce this impact.

Much of *O. ptunarra*'s habitat has been cleared and the remaining habitat is fragmented, with sites now often surrounded by different land uses such as agriculture or plantation forest. The habitat fragments are vulnerable to negative edge effects such as increased competition, predation risk and parasitism (Herse et al. 2018; Pérez-Rodríguez et al. 2018), which can result in a further decline in biodiversity in the habitat fragment. The new land uses adjacent to the *Poa* grasslands often contain highly disturbed ground due to the removal of vegetation and tilling of the soil to plant new crops or trees. Although vespid wasps are known to favour disturbed ground for building nests within plantation forests, the impact of the spillover of wasps into adjacent *Poa* grasslands has not been confirmed. Additionally, while buffers around areas of habitat are often used to protect other threatened species, they have not yet been explored for *Poa* grasslands containing *O. ptunarra* populations.

The present study used GIS to investigate the proportion of vegetation types surrounding the *Poa* grasslands, and whether the vegetation types were influencing numbers of *O. ptunarra* or vespid wasps, and to determine an appropriate buffer size around areas of *O. ptunarra* habitat to limit incursions by vespid wasps.

The fragmentation of *O. ptunarra*'s habitat has also resulted in sites being further apart than previously and separated by unsuitable habitat. *O. ptunarra* is a not a strong flyer and the probability of recolonising isolated sites where populations have become extinct is low (Neyland 1993). Consequently, *O. ptunarra* is vulnerable to stochastic risks. Additionally, the spillover of introduced, predatory vespid wasps from adjacent plantation forests is of particular concern with regard to *O. ptunarra*, due to the risk of local extinctions caused by high levels of predation. Translocation has been suggested in the Recovery Plan for the species as a method of restocking *O. ptunarra* sites that are unable to be naturally recolonised but this conservation action was not implemented before the present study (Bell 1999). The present study investigated the floral requirements of *O. ptunarra* to determine appropriate translocation sites, and also conducted translocation trials to establish new populations of *O. ptunarra* within its historical range.

1.4. Aim and objectives

The aim of this work was to determine the impacts of invasive vespid wasps on *O. ptunarra* and methods for mitigating this threat for the persistence of the butterfly. The objectives of the present study were to: assess the impact of predatory vespid wasps on *O. ptunarra* and determine whether wasps could be effectively controlled to protect *O. ptunarra* populations; analyse the floral composition of the grasslands to determine *O. ptunarra*'s requirements; to learn how to successfully translocate *O. ptunarra* to start new, self-sustaining populations; determine the extent of the vegetation types surrounding the grasslands at various buffer distances and whether they have a significant relationship with *O. ptunarra* or vespid wasp abundance; and determine an optimal buffer zone width around the grasslands to protect *O. ptunarra* from impacts from vespid wasps.

The aim and objectives were achieved by:

i. performing wasp control trials to determine whether vespid wasps were having an impact on numbers of *O. ptunarra* in the northwest grasslands and whether they could be successfully controlled to an extent that protected *O. ptunarra* (Chapter 3);
ii. performing vegetation transect surveys at *Poa* grassland sites both with and without *O. ptunarra* to determine the floral composition of the grasslands and to assess *O. ptunarra*'s vegetation requirements (Chapter 4);
iii. translocating *O. ptunarra* imagoes and eggs to new *Poa* grassland sites within the species' historical range to start new, self-sustaining populations (Chapter 4);
iv. performing a GIS analysis to determine the extent of the vegetation types surrounding the grasslands at various buffer distances and then a statistical analysis to determine whether they have a significant relationship with *O. ptunarra* or vespid wasp abundance (Chapter 5);
v. performing correlation analyses between significantly related vegetation types and average numbers of *O. ptunarra* and vespid wasps, at various buffer distances, to determine an optimal width for buffer zones around the grasslands to protect *O. ptunarra* from impacts by vespid wasps (Chapter 5).

Chapters 3-5 are published papers or papers in preparation. There may therefore be some overlap in material between introductions and with chapters one and two.

Chapter 2 *Oreixenica ptunarra review*

2.1 Description

O. ptunarra is within the Nymphalidae family, in the subfamily of Satyrinae (brown butterflies). The most closely related species to *O. ptunarra* is *O. latialis* Waterhouse & Lyell, 1914 (small alpine xenica) which occurs on mainland Australia in Victoria and New South Wales (Braby 2000). It is also closely related to the two other *Oreixenica* species in Tasmania*, O. lathoniella* Westwood, 1851 (silver xenica) and *O. orichora* Meyrick, 1885 (spotted alpine xenica) but is present later in the season (Braby 2000).

O. ptunarra is a small, orange-brown butterfly that is endemic to the highland *Poa* tussock grasslands of Tasmania. It is a small butterfly species, with a wingspan of approximately 28 mm. The female is brownish-black with orange markings, while the male is brownish-black with cream markings; both are silvery on the underside (Fig. 2.1). When viewed from above, both sexes have four wing spots, one at the apex of each wing. On the underside they have six eyespots, one on each forewing and two on the hindwing (Braby 2000). The species is variable in size and colour, with smaller, darker specimens occurring in the colder northwest uplands and larger, brighter specimens occurring in the warmer south and east of the state (McQuillan and Ek 1997).

Fig. 2.1 The female (left) and male (right) showing colour dimorphism between sexes of *O. ptunarra* butterflies (photos: Simon de Salis)

The eggs are grass green, round and tiny (about 0.75 mm in diameter) with very fine longitudinal ribs (Anderson 2010; Couchman 1953) and are non-sticky (Neyland 1993). The eggs are laid between February and April, either individually on the wing, or in batches when perched on the grass. About four eggs are laid per batch on average, totalling around 26 eggs altogether (Anderson 2001). The eggs incubate for a period between 17 days in the laboratory (Anderson 2001) and six weeks in the wild (Couchman 1953) and then hatch into small larvae (about 2 mm long), with a straw yellow-green coloured body covered in scattered, long, brown hairs and a light khaki-green head (Anderson 2010).

The larvae of *O. ptunarra* are typical of many Satyrinae (Braby 2000) (Braby, 2000). The fully-fed larva, while larger, is still small, varying from about 19 mm long and 4.5 mm wide (Couchman 1953) up to approximately 30 mm long (Anderson 2010). The mature larva has two distinct colour morphs and can be either green or brown (Anderson 2010). The green morph has a grass-green body with an olive-green head and there are narrow lines on the sides and back which are olive-brown and cream (Couchman 1953). The brown morph has a pinkish-brown body with a terracotta brownish-red head and the narrow lines on the sides and back are dark reddish-brown and pink (Anderson 2010). In both morphs the body tapers towards each end and the posterior end is forked (Couchman 1953), the small head is distinctly separated from the thorax, and the body is covered in short, light-coloured, bristle-like setae (Anderson 2010).

The fresh pupa is small and greenish-grey flecked with black, with a pair of black spots on each body segment (Couchman 1953). When mature, the pupal shell is transparent and light brown and the dark spots are raised (Anderson 2010). The pupa measures about 9.5 mm long and 3.5 mm wide at the level of the wing covers (Couchman 1953) and no cocoon is produced (Anderson 2010). The pupa remains loose and unattached, supported in a fork of grass at the base of the tussock (Anderson 2010; Couchman 1953) until the imago emerges.

O. ptunarra is one of the most recent Australian Satyrinae butterflies to be described and among the most restricted in its geographical range. Couchman (1953) defined three allopatric subspecies: *ptunarra*, *roonina* and *angeli*, on the basis of geographically related differences in the size and colour-pattern of individuals from a limited number of sites. However, following the discovery of further populations, particularly in the northwest, it became apparent that not all populations could be confidently assigned to these subspecies. McQuillan and Ek (1997) further analysed geographical variation in the phenotype of males to propose that all three subspecies *angeli, roonina* and *ptunarra* be reduced to the nominate subspecies *O. p. ptunarra.* They also suggested that the isolated populations from the montane grasslands of north-western Tasmania should be recognised as a new subspecies, *O. p. north-west.* However, as the north-west populations largely represent a clinal variation correlated with the change in environmental conditions, further genetic analysis and a formal revision of the taxonomy is required before this subspecies is recognised. Currently, all of the populations are referred to and managed as *O. ptunarra*.

2.2 Lifecycle

The brief flight season of adult *O. ptunarra* butterflies occurs in autumn between the final week of February and the beginning of April and lasts between two and three weeks at any given site. Butterflies at higher altitudes emerge from the pupae before those at lower altitudes (Neyland 1993), possibly due to the earlier emergence of flowers at the higher altitude grasslands. Males emerge before females, with females being mated soon after emergence. The majority of females are monandrous and mate only once (Anderson 2010), shortly after which the eggs are dropped individually into grass tussocks by butterflies on the wing (Neyland 1993), or in batches by butterflies perched on the grass (P.B. McQuillan pers. comm.). The larvae then hatch between 17 days (Anderson 2001) and six weeks later (Couchman 1953) and overwinter in the tussocks, possibly undergoing diapause during the first instar (Anderson 2001). The larvae sporadically emerge at night to feed on the tips of the *Poa* tussock grass (Anderson 2010). In early February pupation takes place, lasting four to five weeks, after which the imagoes emerge and the cycle begins again. *O. ptunarra* only has one generation per year (Couchman 1953).

During the flight season, large numbers of butterflies are present on warm, sunny days with little wind (Neyland 1993). Butterfly activity drops quickly if weather conditions become unfavourable due to increased cloud cover, strong wind or rain, when the butterflies seek shelter in the tussocks. The flight season may be prolonged in unfavourable weather conditions, due to energy-saving periods spent sheltering during low temperatures, while good weather appears to reduce the length of the flight season (Bell 1999). Over the course of the season the numbers of butterflies follow a skewed normal distribution, with butterfly numbers increasing rapidly at the start of the season and gradually declining towards the end of the season, at any given locality (Bell 1999). The abundance of adult butterflies also follows a diurnal pattern, with butterflies emerging for flight around 10 am, peaking in numbers in the middle of the day between 11 am and 2 pm and then declining rapidly after about 3:30 pm (Bell 1999). Accordingly, surveys for this species are usually performed on still, sunny days between the hours of 10 am and 4 pm.

O. ptunarra butterflies usually fly amongst the tussocks, in an apparent searching pattern, often turning back on their own path (Bell 1999). The butterflies rarely lift above the tops of the tussocks except in response to disturbance or predation threat. Males have been observed to fly for up to five minutes without rest (Bell 1999), probably while searching for females, while females typically fly for shorter periods, often stopping to bask on tussocks or flowers (Anderson 2001).

O. ptunarra butterflies are generalist nectar feeders that visit a variety of different species flowering in the grasslands and will feed on any available nectar present (Anderson 2001). Flowers that adult *O. ptunarra* butterflies have been most commonly observed feeding upon are the everlasting daisies (*Xerochrysum bracteatum* V. and *X. subundulatum* R. J. Bayer), common herbs in many of the *Poa* grassland sites during the flight season (Bell 1999), as well as native and introduced dandelions (*Taraxacum* spp*.* F. H. Wiggs) (Anderson 2001). At less natural sites and on roadsides the butterflies predominantly feed upon nectar from introduced species that resemble dandelions (*Leontodon taraxacoides* Hoppe & Hornsch and *Hypochoeris radicata* L.*)* and introduced thistles (*Cirsium* spp. Mill) (Anderson 2001).

O. ptunarra has also been observed to feed upon the flowers of a variety of other species including: bluebell (*Wahlenbergia stricta* R. Br.), ivyleaf violet (*Viola hederacea* Labill.*),* white gum *(Eucalyptus viminalis* Labill.*),* teatree *(Leptospermum* spp. J. R. Forst & G. Forst), mountain pinkberry *(Leptecophylla juniperina* subsp*. parvifolia* (R. Br.) C. M. Weiller*),* white flag iris (*Diplarrena moraea* Labill.*)*, pinkbells (*Tetratheca* spp. Sm.), native cranberry (*Astroloma humifusum* (Cav.) R. Br.), common heath (*Epacris impressa* Labill.), eastcoast everlasting daisy (*Xerochrysum bicolor* (Lindl.) R. J. Bayer), alpine everlasting bush (*Ozothamnus rodwayi* Orchard Var.), other native daisies and introduced species such as gorse (*Ulex europaeus* Labill.) and saffron thistle *(Carthamus lanatus* Labill.*)* (Anderson 2001). Several other species were identified by Anderson (2001) as nectar sources for *O. ptunarra* but are unlikely for the following reasons. The small snowdaisy (*Celmisia saxifraga* (Benth.) W. M. Curtis*)* and cushion plant (*Dracophyllum* sp. Labill.) are unlikely nectar sources for *O. ptunarra* as they are true alpine plant species and occur outside of *O. ptunarra*'s climatic range. The silver wattle (*Acacia dealbata* Link) and black wattle (*Acacia mearnsii* De Wild) are also doubtful nectar sources for *O. ptunarra* as the flowers produce very little nectar; it is more likely that butterflies were simply resting on the plants and not feeding. Some sites do not have obvious nectar sources and it is has been supposed that while nectar intake may enhance longevity and fecundity, it is not necessarily required for survival and reproduction by the imago (Anderson 2001).

The larvae feed on the tips of the *Poa* tussocks and show a preference for *Poa labillardierei* Vickery (var. *labillardierei* and var. *acris*) in the field (Bell 1999) as well as *P. hiemata* Vickery in the laboratory (Anderson 2010). The species has also been collected from *P. rodwayi* Vickery and *P. gunnii* Vickery. It is believed that

P. clelandii Vickery, *P. sieberiana* Spreng. and *P. hookeri* Vickery are also likely to be food plants, as they also occur at sites with *O. ptunarra* (Neyland 1991). Other potential hosts at sites occupied by *O. ptunarra* are *P. hiemata*, *P. fawcettiae* Vickery and *P. costiniana* Vickery.

There are not many documented observations of predation on *O. ptunarra*. However, a small web-spinning spider and introduced vespid wasps (*Vespula germanica)* have been observed to frequently catch and eat the adult butterflies (Bell 1999). Swallows and martins (*Hirundo* spp.) have also been observed swooping into grasslands to catch adult *O. ptunarra* (P.B. McQuillan pers. comm.).

2.3 Distribution and habitat

The ptunarra brown butterfly is endemic to Tasmania and is restricted to highland sites above 450 m with a significant cover of *Poa* tussock (usually greater than 25%). *O. ptunarra* is found across five bioregions within the state, with the majority occurring in the Central Highlands and South East bioregions and fewer occurring at the boundaries of the Southern Ranges, Northern Slopes and Northern Midlands bioregions, based on IBRA Version 7 (DSEWPaC 2012). Habitats occupied by *O. ptunarra* include: Lowland *Poa labillardierei* grassland (GPL), Highland *Poa labillardierei* grassland (GPH), Highland grassy sedgeland (MGH), Eastern alpine heathland (HHE) and *Eucalyptus rodwayi* forest and woodland (DRO). These habitats were previously described by Neyland (1992) using an out-dated classification system, here they have been made consistent with the current standard for vegetation communities in Tasmania (Harris and Kitchener 2005a). The habitat conditions that *O. ptunarra* is associated with are fragmented and likely to be experiencing edge effects.

Habitat modelling and on-ground surveys performed prior to 1999 indicated there were 150 discrete colonies of *O. ptunarra* (Neyland 1993) occupying an area of approximately 13,900 ha (Threatened Species Unit 1998) and that *O. ptunarra* then occupied its full potential range with the possible exception of areas of the eastern highlands and western Central Plateau, which have suitable habitat but where *O. ptunarra* was not detected (Neyland 1993). These areas have been extensively but unsuccessfully surveyed in the hope of detecting *O. ptunarra*, so it is possible that they have become locally extinct from these areas, or never occurred there (P. Bell pers. comm.). Additionally, some seemingly ideal sites did not have populations of the butterfly, such as the lowland plains of the Midlands where it is believed to be too warm for the butterfly to flourish (Threatened Species Unit 1998). It has also been claimed that the lowlands may be too dry for the *Poa* to thrive (Threatened Species Unit 1998) but this is not the case as there are still remnants of healthy lowland *Poa* grasslands in the Midlands (Fensham and Kirkpatrick 1989). Other seemingly ideal sites may be lacking populations of *O. ptunarra* due to a history of over-grazing or inappropriate fire regimes (Threatened Species Unit 1998) or stochastic losses.

Overall, *O. ptunarra* has undergone a substantial reduction in area of occupancy since European settlement. Much of the original habitat for *O. ptunarra* has been lost, as around 40% of the original area of native grassland in Tasmania was cleared in the period 1802-1995, the greater part of which was *Poa* grassland (Kirkpatrick et al. 1995). In the Midlands, less than 3% of the original extent of native grasslands remains intact (Fensham and Kirkpatrick 1989). The majority of the grasslands were converted to cropland, pasture and plantation forest (Bell 1999). *O. ptunarra* is absent from these converted areas, with small populations now sometimes found on the fringes of areas which once would have supported large colonies (Neyland 1993). It is likely that some sites have also undergone a reduction in population size since 1999 due to an extended drought in the Midlands (2001-2008) and overgrazing, losses due to land use change from grazing to cropping and losses resulting from a recent surge in on-farm dam development (P. Bell pers. comm.).

O. ptunarra ranges across the Central Plateau, the Midlands, the Eastern Highlands and northwest Tasmania. On the Central Plateau the butterfly is scarce in the more elevated areas and only occurs patchily in a range of grassy communities dominated by *Poa gunnii, Hakea microcarpa* R. Br. or *Richea acerosa* (Lindl.) F. Muell. On the lower plateau surface near the Steppes there are large areas of suitable habitat. Here *O. p. ptunarra* is usually found in *Eucalyptus rodwayi* R. T. Baker & H. G. Sm. forest and woodland (DRO) in grassy open areas (Bell 1999). In the Midlands, populations of the butterfly are found only in *Poa labillardierei* tussock grasslands in the more elevated areas (Bell 1999). Prior to European settlement the Midlands was a mosaic of grasslands, woodlands and open forests, but about 90% of natural habitats have now been converted to improved pasture (Fensham and Kirkpatrick 1989) leaving little habitat for the butterflies. In the Eastern Highlands *O. p. ptunarra* is found mainly in poorly drained areas which support grassy and/or sedgy communities often dominated by *Eucalyptus rodwayi* and sometimes by *E. pauciflora* Sieber ex Spreng. (Bell 1999). In northwest Tasmania *O. ptunarra* occurs in the highland *Poa* grasslands, where a combination of frosts, fire and marsupial grazing have created a mosaic of grasslands, eucalypt forests and rainforests. Here, the butterfly is usually found in grassy open woodland dominated by either *Eucalyptus rodwayi* and/or *E. delegatensis* R. T. Baker, open grasslands dominated by *Poa labillardierei,* or grassy shrubland dominated by *Hakea microcarpa* (Bell 1999).

Most of the information regarding populations of *O. ptunarra* which occur on reserved land is from the late 1990s and has not been updated since. In 1998, only 6 of the 150 known populations of *O. ptunarra* were protected within the Tasmanian Wilderness World Heritage Area, comprising about 600 ha within the Central Plateau Protected Area and 50 ha within Cradle Mountain Lake St Clair National Park (Threatened Species Unit 1998). In 1999, only about 6% of the known range of the species occurred within reserved land, with approximately 76% of the total population occurring on private land and a further 18% on other State-owned land, including State Forest and Hydro Electric Commission land (Bell 1999). In 1995, only approximately 2% of the total remaining area of *Poa* grassland was reserved in Tasmania, representing a total area of 1,183 ha of *O. ptunarra* habitat (Kirkpatrick et al. 1995). Populations and habitat of *O. ptunarra* that occur within reserved land are considered to be protected if the reserves are managed appropriately.

Several areas containing *O. ptunarra* populations have recently been placed under private Conservation Covenants and Management Agreements, adding them to the Tasmanian Reserve Estate. Current information on the Natural Values Atlas online database indicates that 26% of *O. ptunarra* records presently occur within reserves, the majority of which are within private reserves, with only 9% of the records occurring within public reserves (DPIPWE 2019).

2.4 History of conservation and recovery planning for *O. ptunarra*

By the 1980s it was recognised that the ptunarra brown butterfly was in decline, mainly due to habitat loss and fragmentation. In 1988 only 33 locations were known for the butterfly and the species was considered to be threatened (Prince 1988). The recovery process began in 1986 with government funded research assessing the butterfly's habitat requirements and conservation status, followed by further research in 1990, which culminated in a Conservation Research Statement (Neyland 1992). The Statement reported 117 additional colonies all of which were to some extent threatened, and subsequently all of the subspecies of the butterfly were considered to be endangered based on IUCN criteria (Wells et al. 1983). The number of colonies located further increased to 150 in 1993 (Neyland 1993) and all are now believed to have been located. *Oreixenica ptunarra* was consequently listed as vulnerable in Schedule 4 of the Tasmanian *Threatened Species Protection Act 1995* (Bell 1999) and as endangered under the federal *Environment Protection and Biodiversity Conservation Act 1999*.

The historical range of *O. ptunarra* was once widespread across central Tasmania in grassland, grassy shrubland and open, grassy woodland habitats containing *Poa* (Threatened Species Unit 1998). However, there has been a large decline in habitat for the species since European settlement, largely due to land clearing (Kirkpatrick et al. 1995). The cleared *Poa* grasslands were generally converted to cropland, pasture and plantation forest. Due to this loss and the ongoing decline of the grasslands, the highland *Poa* grasslands are now listed as a threatened native vegetation community under the Tasmanian *Nature Conservation Act 2002,* while the lowland grasslands of Tasmania, which include Lowland *Poa labillardierei* grasslands (GPL), are listed as critically endangered under the federal *Environment Protection and Biodiversity Conservation Act 1999*. This provides important protection for areas of *O. ptunarra* habitat as they cannot be cleared or converted without approval from the regulating government body.

Following the listing of *O. ptunarra* as vulnerable, a recovery plan was developed for the species (Neyland 1991). Objectives of the plan included identifying previously unknown areas of butterfly habitat and undertaking a program of education and initiate co-operative management of butterfly habitat. These were successfully implemented (P. Bell, pers. comm.). Other objectives, such as monitoring and reporting, are ongoing, while the objective to establish new colonies in suitable areas through translocation was rolled over into the current recovery plan without any implementation.

The current *Ptunarra brown butterfly recovery plan 1998-2003* is overdue for revision. It proposes 'to achieve down listing of *O. ptunarra* to a lower risk category within five years, based on the IUCN (1994) criteria of population size and trends, area of occupancy and security of habitat' (Bell 1999). The aim is to achieve this by: increasing habitat protection for specific populations, ensuring the long-term persistence of the species throughout its area of occupancy, providing advice and information to land owners and managers, establishing a new colony by translocation, clarifying the taxonomy, and assessing the conservation status of *O. ptunarra* subspecies (Bell 1999).

The current strategy for the species' recovery is based on the taxonomy proposed by McQuillan and Ek (1997). It is therefore important that this taxonomy is formally recognised as soon as possible. Anderson (2010) attempted to elucidate the genetics of the species, but the sample size was too small and consequently the results were uncertain. Further research is required to clarify the taxonomy of *O. ptunarra*.

As most of the populations of *O. ptunarra* are contained on private land and only a small proportion are found within formal reserves and other Crown land, the long-term future of the species relies on the sympathetic management of habitat by private land owners. Therefore, the development of management agreements with land owners and/or land managers is important for the long-term security of the butterfly. A good example of how such agreements have been successful is in the Northwest Plains where less than 2% of the known habitat of *O. ptunarra* occurred within formal reserves in 1999 (Bell 1999) and over 80% of the populations occurred on land owned by a single private timber company, representing over 50% of the area of habitat. By working closely with this company it was possible to secure habitat for six populations of *O. ptunarra* by initially including areas within the company's private reserve system (Bell 1999) and later by placing conservation covenants on several key sites, which added these areas to the State's reserve estate and, importantly, protected them in perpetuity (P. Bell pers. comm.). Agreements with landowners could equally be sought for specific populations in the Midlands and in the north east of the butterfly's range (Bell 1999). Many of the *O. ptunarra* populations in this area are now managed by patch-burning the grasslands at an appropriate rate to maintain them at an early successional stage with sufficient gaps between the grass tussocks for herbs to grow and few woody shrubs.

Neyland (1993) recommended burning *O. ptunarra* habitat on a seven-year cycle in a mosaic style, in which different areas are burnt each year. Similarly, the Tasmanian government's Threatened Fauna Handbook recommends cool, low-intensity fires on a rotation of 5-10 years in a mosaic pattern (Bryant and Jackson 1999). Anderson (2001) found that patch burning on a five to six year rotation is the prevailing burning regime in the Tasmanian Midlands and suggests that this is suitable for the management of *O. ptunarra* in this area. These fire frequencies reduce fuel loads while maintaining the grassiness of the site and also promote growth of herbs between the tussocks (Neyland 1993). Too frequent fires will lead to decline in *Poa* and an increase in shrubs and consequently a decline in *O. ptunarra* population. For example, Neyland (1993) found that sites burnt at a frequency of every three years or less have had reductions in butterfly numbers.

Fires in late autumn to winter are recommended, as the larvae are likely to be sheltering deep within the tussock during this time and are less likely to be killed by a cool burn that just burns the outside of the tussock (Neyland 1993). Fires during the adult flying season (Feb – April) are not recommended as the adults may be killed before they have an opportunity to lay their eggs, which may considerably reduce the population at the site of the fire (Bryant and Jackson 1999). Spring fires are also generally not recommended, as the larvae are feeding on the *Poa* during this time and may be killed by the fire, or may starve or have impaired growth if there is insufficient food left following the fire (Bryant and Jackson 1999).

Few *O. ptunarra* are found on sites which are heavily grazed or frequently burnt (Neyland 1993), as much of the *Poa* tussock has been removed and not much food or shelter remains for the butterflies and their larvae. If large swathes of the landscape have been burnt then there is potential for local populations to be extirpated, preventing recolonisation of burnt areas. Conversely, in grasslands where fire and grazing are absent, butterfly numbers are also usually low, as the tussocks often become large and overgrown, with few inter-tussock spaces for butterflies to fly or for herbs and flowers to grow (Neyland 1993). In these instances, the grassland may also gradually shrink due to invading shrubs and trees (Bell 1999). Patch burning is the preferred method for the management of grasslands containing *O. ptunarra* as some of the local population will survive to recolonise the burnt areas of the site.

In the Midlands, light grazing has assisted in managing some *O. ptunarra* habitat that may have otherwise become overgrown. Grazed tussock grasslands were favoured by *O. ptunarra* over ungrazed areas, or weed infested tussocks on roadsides (Bell 1999). Grazing by sheep is preferable to grazing by cattle, as the cattle can pull out bigger tufts of grass and sometimes the whole tussock, which can lead to the death of the tussock and potentially the decline of the grassland (Anderson 2001). Cattle grazing during the *O. ptunarra* pupation and flight period (January to mid-April) should be avoided where possible (Anderson 2001) as the cattle may consume pupae or adults sheltering within the tussocks. Butterflies are sometimes at risk of being trampled by stock.

For sheep grazing, a light set stocking rate of around 2-3 DSE (dry sheep equivalent) per hectare is recommended for maintaining healthy *Poa* tussocks which will benefit *O. ptunarra* populations (Anderson 2001). Heavy grazing can cause areas of bare ground, which encourages weed invasion and high stocking rates can also mean high levels of fertiliser produced by the stock, which causes nutrient enhancement of the soil, promoting the growth of weeds (Kirkpatrick 1991). Small *O. ptunarra* colonies that are located within large areas of pasture would benefit from being fenced off and occasionally grazed or burnt to protect the colony from overgrazing (Anderson 2001; Bryant and Jackson 1999).

The practice of cell grazing, where many sheep heavily graze a small area for several weeks before being moved to the next cell, leaving the grazed area to recover, is being used more often by farmers in the Midlands (Anderson 2001). This practice encourages grazing of all the grass species present in the cell, not just the preferred pasture species, and allows the cell time to recover between bursts of grazing. Cell grazing regimes can be detrimental to *O. ptunarra* colonies if the entire habitat in the area is heavily grazed at once, as all the food for the larvae may be removed. However, if only patches of habitat are contained within each cell, then the cell grazing acts like mosaic burning and can be an efficient way of managing habitat for the benefit of *O. ptunarra* (Anderson 2001).

Most farmers with populations of the butterfly on their property were contacted during the development of the original recovery plan from 1992-1997. In response to the recovery program some farmers reduced the size of their sheep flocks in butterfly habitat and/or considered the requirements of the butterfly as part of their farm management practices (Bell 1999). As the majority of *O. ptunarra* sites are on private land, with only about 9% contained within public reserves, the long-term security of the species depends on ongoing liaison with land owners and developing agreements for the sympathetic management of butterfly habitat (Bell 1999).

In order to encourage landowners to include *O. ptunarra* in the management of their properties, up to date information about the species must be regularly provided to the public. A brochure detailing the biology and conservation of *O. ptunarra* was prepared in 1996 and distributed to landowners, councils and other interested groups and individuals within the area of occupancy of the butterfly. The brochure has since been superseded by the listing statement (Threatened Species Unit 1998), which is intended to be intermittently updated and is available from the DPIPWE website (Appendix A).

Anderson (2001) found that most landowners interviewed in the Midlands about butterfly management were not aware of the existence of *O. ptunarra* on their properties, even though many farmers had previously been made aware of the butterfly during the recovery program and by the brochure (Bell 1999). Nevertheless, many landowners indicated that they would consider *O. ptunarra* in their future management plans if they had the relevant information (Anderson 2001).

Climate change is a threat to *O. ptunarra* that has not been considered to any great extent. A change in grassland distribution due to climate change may result in a reduction of available habitat for *O. ptunarra*, or a change in the habitat's location which the butterflies may be unable to find due to their weak flight and the fragmentation of their habitat. Climate change may also promote the invasion of woody vegetation onto grasslands through an increase in minimum temperatures causing a decline in frost incidence and intensity (Hughes 2003). There may also be a changes in fire frequency, due to an increased occurrence of dry lightning (Styger et al. 2018), which may change the floral composition of the grasslands or cause extirpations of butterfly populations. Further research and modelling are required to determine the impact of climate change on *O. ptunarra* and how to mitigate these impacts.

Agricultural chemicals such as fertilisers, herbicides and pesticides also pose a threat to butterflies and are regularly used in Australia, including on paddocks and crops adjacent to *O. ptunarra* colonies in the Tasmanian Midlands (Anderson 2001). Herbicides and pesticides are also often used in plantation forests and may impact on *O. ptunarra* populations if there is any spray drift across the grassland (Neyland and Brown 1996). In the Midlands, chemical usage is generally moderate and most sites only use super-phosphate fertiliser and one or two additional chemicals, but cropped sites sometimes use more than three chemicals (Anderson 2001). Anderson (2001) suggested that these chemicals did not appear to be having an adverse effect on *O. ptunarra*, but further investigation is warranted, focusing particularly on the larval stage.

Building habitat corridors between sites has been suggested as a way of reconnecting fragmented populations (Neyland 1993) but this method has not been implemented so far, presumably because of the cost and practical difficulties. The possibility of translocating *O. ptunarra* to combat stochastic effects within the fragmented landscape was investigated during the period of the first recovery plan but not implemented. It was intended to collect butterflies from several areas in the Northwest Plains destined for destruction and to translocate them to two large areas of suitable habitat where the butterfly had not been previously recorded. However, due to several new colonies being discovered in the Northwest Plains and the ethical difficulties in introducing the butterfly to new areas, no translocations were undertaken (Bell 1999). The current recovery plan also suggests that translocation may also be a useful conservation tool to protect *O. ptunarra*, should it become necessary to boost population numbers or utilise presently unused habitat, or in instances where *Poa* reinvades cleared land or improved pastures.

The new threat of introduced vespid wasps predating on *O. ptunarra* and potentially causing a decline in their numbers is considerable and was not investigated before the present study. Most wasp control trials in Tasmania have been to protect crops and workers by means of toxic baits (Bashford 2010; Statham 2001).

The present thesis extends the knowledge of the interaction *O. ptunarra*, its environment and predatory vespid wasps in the hope of providing better guidelines for recovery, as identified in Chapter 1. The next chapter addresses the interactions between *O. ptunarra* and vespid wasps in the *Poa* grasslands of north-western Tasmania, determining the effectiveness of wasp control in maintaining butterfly populations.

Chapter 3 The effects of introduced vespid wasps (Vespula germanica and V. vulgaris) on threatened native butterfly (Oreixenica ptunarra) populations in Tasmania

The following chapter is a marginally amended version of the publication: Potter-Craven J, Kirkpatrick JB, McQuillan PB, Bell P (2018) The effects of introduced vespid wasps (*Vespula germanica* and *V. vulgaris*) on threatened native butterfly (*Oreixenica ptunarra*) populations in Tasmania. J Insect Conserv 22:521-532. https://doi.org/10.1007/s10841-018-0081-9

Abstract

Introduced vespid wasps (*Vespula germanica* and *V. vulgaris*) are highly efficient predators of native invertebrates. They have the potential to reduce populations of threatened species and change ecosystem dynamics, yet their impact is largely unknown in Australia. The introduction of vespid wasps has coincided with a decline in numbers of threatened ptunarra brown butterflies (*Oreixenica ptunarra*) in Tasmania, Australia. The ptunarra brown butterfly is endemic to Tasmania, where its habitat has been fragmented by clearance for agriculture and forestry. Local extinctions of the species were previously thought to be principally due to its inability to fly the long distances between habitat patches in this disjointed landscape. We investigate the importance of the new threat of vespid wasp predation in the decline of *O. ptunarra* in the highland grasslands of northwest Tasmania. Numbers of *O. ptunarra* analysed over a period of 15 years dramatically declined after the arrival of vespid wasps. Wasp control was trialled to determine whether it affected butterfly numbers. Current control methods decreased wasp numbers considerably, resulting in a small increase in butterfly numbers, indicating that wasp predation is keeping *O. ptunarra* at low densities. Without ongoing conservation measures, it is likely that butterfly numbers will stay low, potentially leading to genetic bottlenecks and more local extinctions. An increase in the intensity of wasp control, in combination with other conservation management methods, is required for the protection and recovery of *O. ptunarra*.

3.1 Introduction

Invasive species are increasingly becoming an environmental problem due to increasing volumes of global trade and the movement of people. Many invasive species flourish in new environments and then outcompete or predate upon native species, which can cause a change in species composition, niche shifts and local extinctions (Eastwood et al. 2007). Invasive species are the second greatest threat to endangered species in the USA, behind habitat loss (Wilcove et al. 1998), with social insects such as wasps and ants being amongst the most concerning, as they are able to proliferate quickly and are usually generalist predators, enabling them to rapidly have a large impact on native species.

Vespid wasps are an introduced pest in countries such as New Zealand and Argentina, where they have a significant negative impact on native species (ie. Beggs 2001; Harris and Oliver 1993; Masciocchi and Corley 2013; Sackmann et al. 2008; Toft and Rees 1998). *Vespula* queens often overwinter in human goods and are believed to have been recently widely disseminated via global trade in shipping containers (Beggs et al. 2011). Establishment can occur quickly as only one fertilised queen is necessary to start a new population (Beggs et al. 2011; Spradbery and Maywald 1992).

The Palaearctic European wasp (*Vespula germanica*) and English wasp (*V. vulgaris*) are recent arrivals in Australia. *V. germanica* was first detected in Hobart, Tasmania in 1959. It is believed to have arrived in cargo from New Zealand (Spradbery and Maywald 1992). It spread at a rate of 60–70 km/year to become widespread throughout the island by 1974 (Spradbery and Maywald 1992) with the last of the marginal rainforest habitat becoming occupied by 1993 (Bashford 2001). *V. germanica* prefers to colonise open or partly shaded sites (Bashford 2010) and is now present in Tasmania in all major vegetation types except mountain moorlands (Spradbery 1973a). Genetics show that the colonisation of mainland Australia by *V. germanica* 15 years later was not via Tasmania but through a separate introduction from overseas (Goodisman et al. 2001).

Vespula vulgaris became established in Tasmania in 1995 (Matthews et al. 2000) and spread to the south east of the State, including Hobart by 2000 (Bashford 2001), and to the central north by 2010 (Bashford 2010). Matthews et al. (2000) suggest that the additive effect of the recent invasion of a second species of predatory wasp in Tasmania could be ecologically profound.

The only native social wasps that occur in Australia in the family Vespidae are in the subfamily Polistinae, which are the paper wasps. Many native paper wasp species provision their nests with larval Lepidoptera. Australia does not have any native wasp species in the subfamily Vespinae which includes *Vespula* spp., such as the European and English wasps. Neither paper wasps nor vespid wasps occur naturally in Tasmania, making the island particularly vulnerable to invasive introduced vespids.

The diet of vespid wasps is high in carbohydrate sugars and protein. The carbohydrate sugar sources such as nectar, honeydew and fruit are used primarily for their own energy requirements, while the protein, which is largely from invertebrate prey but also scavenged from the carcasses of larger animals, is taken back to the nest (Spradbery 1973b). Introduced vespid wasps are known to cause damage to ecosystems through predation of local invertebrates as well as through competition with local invertebrates for food, sometimes leading to the starvation of other species (Burne et al. 2015).

In the *Nothofagus* Blume forests of New Zealand, vespid wasps feed voraciously on the carbohydrate rich honeydew produced by scale insects *Ultracoelastoma assimile* (Maskell) on the trees, booming to huge numbers, after which they both consume and outcompete native invertebrates for this resource (Brockerhoff et al. 2010). They sometimes also outcompete vertebrates such as birds and have disrupted long co-evolved bird–honeydew associations (Gardner-Gee and Beggs 2013). *V. vulgaris* has been observed eating large numbers of dispersing winged *Prolasius* ant queens in New Zealand, with the ants sometimes comprising > 25% of the wasps' diet (Burne et al. 2017). In areas where wasp densities are high, this level of predation can erode genetic diversity, leading to a genetic bottleneck (Burne et al. 2017).

Vespid wasps are also known to predate upon butterflies and their larvae. In New Zealand, one of the most common prey items eaten by vespid wasps in the scrubland-pastures are lepidopterans (Harris and Oliver 1993), with predation levels high enough to reduce butterfly numbers (Beggs 2001) and restructure lepidopteran communities (Beggs and Rees 1999). Therefore, there is clearly the potential for invasive vespid wasps to increase the likelihood of extinction of rare or threatened butterflies that share their habitat.

Wasps can be killed by directly poisoning the nests, toxic baiting and biological control. However, their numbers must be reduced to a very low level for the control to be effective (Beggs and Rees 1999; Toft and Rees 1998). Wasp control trials have been widely performed for both conservation and agricultural purposes (see Beggs 2000; Beggs et al. 1998; Hanna et al. 2012; Sackmann et al. 2008; Toft and Rees 1998). In Australia wasp control has been performed primarily for the protection of agricultural crops (Lefoe 2001; Statham 2001) and the protection of workers during forestry operations (Bashford 2010) but very little has been undertaken for conservation purposes. Wasp control is often undertaken in areas where wasp numbers are high, or where they have an impact on local invertebrates or crops. Vespid wasps are known to damage fruit crops in Tasmania and can cause production losses of up to 25% in vineyards and 20% in strawberry fields (Bashford 2001). Wasps may also impact on the honey industry by robbing beehives and predating upon bees (Bashford 2001; Crosland 1990; Madden 1981). They can also have a negative impact on tourism (Masciocchi and Corley 2013).

There have been very few studies quantifying the direct impacts of introduced wasps on native species and ecosystems in Australia. In Tasmania, Spradbery and Maywald (1992) observed that areas containing high numbers of *V. germanica* had been denuded of arthropod prey, resulting in a severe local reduction in native invertebrates, such as butterflies. However, this impact has not been quantified.

The ptunarra brown butterfly (*Oreixenica ptunarra*) is a small orange-brown satyrine that is endemic to the highland *Poa* tussock grasslands of Tasmania. The host plant for the species is *Poa* grass (Neyland 1993). The eggs are laid directly into the *Poa* tussocks, the caterpillars live and feed upon the *Poa* grass and the cocoon is concealed within the tussock. When the butterflies emerge they are generalists that consume nectar from the assortment of flowers in bloom on the grasslands and around the margins, during their flying period (Anderson 2001). *O. ptunarra* is listed as vulnerable and endangered under Tasmanian and Australian government legislation respectively, primarily due to habitat loss through land clearing, fragmentation of habitat, and unsuitable fire and grazing regimes (Bell 1999). The decline in butterfly numbers and their inability to recolonise areas from which they have disappeared, due to their low vagility and the fragmentation of the landscape, is of concern as it may lead to the eventual extinction of the species. Recently, predation by introduced vespid wasps has been recognised as a new threat to *O. ptunarra* (Bell 1999). This new pressure on butterfly numbers could be the breaking point for the species.

We undertake the first investigation of the degree of impact of the introduced vespid wasps *V. germanica* and *V. vulgaris* on the threatened ptunarra brown butterfly. We hypothesise that (i) predation by vespid wasps can reduce populations of the ptunarra brown butterfly, and that (ii) wasp control by poisoning can reverse this decline.

3.2 Methods

3.2.1 Study sites

The present study was conducted at nine sites in northwest Tasmania, Australia near the township of Waratah (41.446°S 145.532°E, 600 m asl) (Fig. 3.1). Populations of vespid wasps and *O. ptunarra* were present at all nine sites. Vegetation at all of the sites was highland *Poa labillardierei* grassland (Harris and Kitchener 2005b). Flowering dicotyledonous herbs, whose nectar is consumed by adult butterflies, were also present in varying abundance. All of the sites occurred in the privately owned Surrey Hills area. They were interspersed between forestry plantations, clear-felled ground and remnants of native forest vegetation. Some of these grassland sites were protected by formal reserves, while others were informally protected by the landowner.

The study area has a cool temperate climate and it receives an average of 2000–2400 mm rainfall/year, with no month receiving < 100 mm (Bureau of Meteorology 2011). The underlying geology of all of the sites is Tertiary basalt (Land Information Systems Tasmania 2014). Native herbivores, mainly wallabies and wombats, grazed all of the sites.

Control of vespid wasps was undertaken at five of the sites, while at the remaining four monitoring sites there were no wasp control activities (Fig. 3.1). The two types of sites were compared to determine whether the control was successful in controlling vespid wasp numbers and whether reduction in wasp numbers affected the number of *O. ptunarra*.

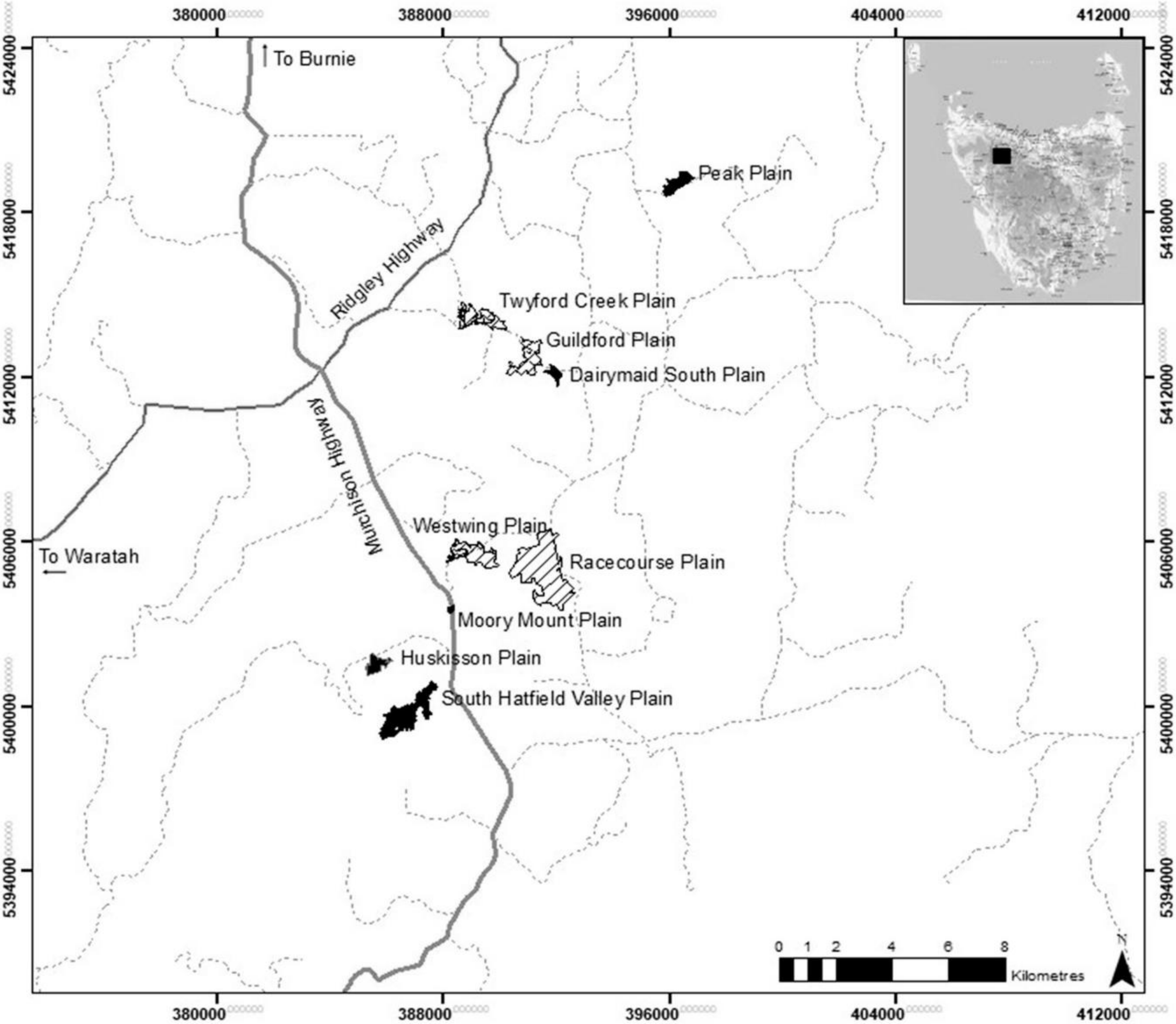


Fig. 3.1 Location of sites showing treatment: wasp control sites (black), non-control sites (striped). Both vespid wasps and *O. ptunarra* were present at all sites

3.2.2 Data collection

3.2.2.1 Wasp and butterfly numbers

Numbers of *O. ptunarra* and vespid wasps were monitored at each site by counting individuals along a fixed transect (Pollard 1977) at approximately weekly intervals during the *O. ptunarra* flight season from early March to early April from 2011 to 2013. All female and male *O. ptunarra* and all vespid wasps sighted within 5 m on either side of the transect line were recorded. Counts were also made up to 5 m in front but no counts were made behind. Every effort was made to only count individuals once and double counting was kept to a minimum. The transects were unsystematically oriented to cover the greater part of the site and varied in length between sites, so butterfly and wasp numbers were standardised by determining how many were present per m^2, from which densities per hectare were calculated. Every effort was made to walk the transects on the day with the most suitable weather forecast for the week in order to maximise butterfly sightings. Transect walks were generally performed between 10 am and 4 pm on sunny, clear days with little wind. However, as weather in the highlands of Tasmania is highly variable, this was not always possible. All observers were trained in identifying *O. ptunarra* and had several years of experience surveying the species.

Butterfly and wasp monitoring surveys were performed during this study in the period of 2011–2013 and have previously been performed in the period 1998–2002 along the same transects, using the same technique as described above. The data recorder for the earlier period (P. Bell) was also present and performed some of the surveys during the later period, which were largely performed by the author (J. Potter-Craven) with the assistance of trained volunteers. The earlier data set was used within this study to enable the comparison of butterfly and wasp abundance during the two time periods to determine any changes in abundance over the 15 year time period. Data for the earlier time periods was only available for five sites (Dairymaid South Plain, Peak Plain, Racecourse Plain, South Hatfield Plain and Thompsons Plain), so comparisons could only be made for these sites.

Wasp numbers were also monitored by counting numbers caught in wasp traps at weekly intervals, from late February to early April in 2011, 2012 and 2013. The trapped wasps were preserved in labelled vials with 75% ethanol and identified to species level in the laboratory. The wasp monitoring traps were generic flying insect traps by Enviro- Safe™ comprising a tall plastic jar with a bright yellow cap and a screw top lid containing funnel holes that the wasps could enter but not exit. The traps were half-filled with a 10% sugar solution, and a cube of processed luncheon meat roughly 2 cm in size was placed in each trap, where it would float at the surface. As wasps are attracted to both sugar and meat, a combination of the two was likely to attract the maximum number of wasps. The monitoring traps were attached to the top of a 1.5 m high wooden stake, or hung from a tree to avoid interference from native mammals.

Wasp monitoring traps were placed at all sites, with a minimum of one trap at each site (Table 3.1). Sites with an area of < 30 ha received one trap, most of the larger sites had two traps, with two of the largest sites, Racecourse and Westwing, receiving three and four traps respectively. The traps were placed by dividing each site into grid squares and then using a random number table to determine the placement locations. At small sites that only had one monitoring trap, the trap was placed in the middle of the site. An additional trap was later added at Huskisson where the randomly placed traps were close together, to get a better indication of wasp numbers elsewhere on the site.

3.2.2.2 Environmental variables

Environmental variables were measured on site before beginning the transect count. Temperatures and wind speeds were measured using a hand-held weather meter (Kestrel 3000, Nielsen-Kellerman, Pennsylvania, USA). The temperature was measured at ground level in the shade in degrees Celsius and the wind speed was measured at chest height, approximately 1.5 m above ground level in metres per second. Time of day was recorded in Australian Eastern Standard Time and adjusted for the daylight saving time change at the start of April each year. Cloud cover was estimated as the percentage of the sky covered by cloud.

Table 3.1 Details of the study sites including size, treatment, trap numbers and control trap density

Plain	Size (ha)	Treatment	No. of monitoring traps	No. of control traps	Density of control traps/ha
Moory Mount	4.0	Control	1	5	1.25
Dairymaid South	22.4	Control	1	9	0.4
Huskisson	34.3	Control	3	11	0.3
Peak	38.7	Control	2	11	0.3
South Hatfield	128.3	Control	2	14	0.1
Twyford Creek	72.3	Monitoring	2	–	–
Guildford	75.7	Monitoring	2	–	–
Westwing	94.9	Monitoring	4	–	–
Racecourse	324.0	Monitoring	3	–	–

3.2.2.3 Spring weather variables

Three additional weather-related variables likely to affect wasp numbers were derived from Bureau of Meteorology records (Bureau of Meteorology 2017) for the previous Spring (Sept–Nov), namely the number of daily minima below 2 °C that would indicate ground frosts, number of extended ground frosts (> 2 days) and the monthly rainfall (actual and average). Spring weather has been determined to influence wasp abundance, as it is a vulnerable time for the queens in establishing their nests after awakening from winter hibernation (Lester et al. 2017; Masciocchi et al. 2016). Nests are still small and the queens initially do not have any workers to feed them, expand the nest and keep the nest warm (Masciocchi et al. 2016). Excessive rain in the spring can flood nests, and ground frosts can chill them, either of which can kill the queen (Madden 1981). The data were collected for the closest town to the study sites that recorded the required information; the rainfall data were collected from Waratah and the temperature data were from Mt Read. Where data points were missing, a regression analysis was performed with an adjacent weather station (Cradle Mountain for rainfall, Luncheon Hill for temperature), which was then applied to the dataset to fill in the gaps. As Mt Read is approximately 500 m higher in altitude than the study sites, the temperature was adjusted using the lapse rate for minimum temperatures in regional Tasmania of 0.37 °C/100 m as determined by Nunez (1988).

3.2.2.4 Vegetation

The vegetation at 15 *Poa* grassland sites in the local area, including the study sites, was surveyed between February and April 2013. *O. ptunarra* were present at ten sites to varying degrees and not present at five sites. The vegetation was surveyed along four separate 10 m transects randomly located along each of the existing butterfly monitoring transects at each site (a total of 60 surveys altogether). The vascular plant species present, the height of each plant, and the presence of bare ground, leaf litter, lichen, moss or scats was recorded at each 0.5 m point interval along the 10 m transect. Everything directly under the point was recorded. Plant species were identified to species level where possible, and otherwise to genus if the reproductive organs necessary for identification were not present. The percentage frequency of occurrence of each vascular plant species present was then determined for each transect.

3.2.3 Wasp control

Vespid wasp control was attempted by two complementary methods, firstly by directly poisoning any vespid wasp nests, and secondly by toxic baiting. Direct poisoning involved subjecting wasp nests to a lethal poison in the form of Permethrin insecticide powder. Permethrin was used as it is a good broad spectrum pyrethroid chemical that is an effective neurotoxin for insects, but has low toxicity for mammals (Toynton et al. 2009). Any vespid wasp nests that were detected at the wasp control sites were immediately poisoned by thoroughly dusting the entrance to the nest. Wasps entering and exiting the nest were exposed to the poison located at the entrance and subsequently carried it further into the nest, thereby poisoning other individuals, as well as larvae and the queen. The nest position was then marked with coloured flagging tape and its co-ordinates recorded. The nests were checked at the next weekly visit to the site to see if they were still active or if they had been destroyed. If some wasp activity was still apparent at the nest, it was poisoned again.

Toxic baiting is currently thought to be the most effective control method for invasive wasps, however, it has been largely experimental and many of the poisons are not widely available (Beggs et al. 2011). The neurotoxic poison Fipronil, a broad-use insecticide that belongs to the phenylpyrazole chemical family, is currently one of the most effective insecticides available for the baiting of wasps, as it is lethal to insects at low concentrations, fast acting and causes minimal harm to mammals that may incidentally ingest it (Hainzl et al. 1998; Harris and Etheridge 2001). Fipronil has previously been used to effectively control vespid wasps in agricultural trials in Tasmania (Bashford 2010; Statham 2001). The poison is disguised within meat, which the wasps collect and carry back to their nest to feed their young. Statham (2001) and Bashford (2010) found that wallaby mince was the most attractive meat bait for *V. germanica* in Tasmania and that it attracted more wasps than the fish or beef baits commonly utilised in other studies (i.e. Pereira et al. 2013; Sackmann et al. 2001; Spurr 1996). Fipronil was used under permit numbers PER9326 and PER13184 issued by the Australian Pesticides and Veterinary Medicines Authority.

A wasp control trap designed locally by Statham (2001) is mammal-proof and was used for the toxic baiting in the present study in order to minimise impacts on the local wildlife, such as possums, quolls and Tasmanian devils. In some studies, bittering agents have been added to baits to deter mammals from consuming them (Sackmann et al. 2010), however, these have also been found to reduce the attractiveness of the baits to the wasps (Statham and Warren 2002).

The wasp control traps were made from two steel tins, joined together by a removable piece of wire, and affixed to the top of a tall wooden stake. The slightly smaller tin containing the bait hung below the other larger tin, with the openings facing each other. The bottom tin had two 10 mm diameter holes drilled in it so that the wasps could easily enter to access the bait. A warning and information label was affixed to the tins detailing the nature of the trap, the poison used, and the phone number to call in case of an emergency. Each trap was affixed to the top of a 1.5 m high wooden stake that had been driven into the ground. The traps were spaced at 300 m intervals around the perimeter of the grassland. This distance was chosen to maximise the number of wasps visiting the trap, as wasps usually do not travel > 400 m from the nest (Archer 2012).

When wasp numbers were noted to increase, either by an increase in wasp numbers counted in wasp monitoring traps or along transects, or by the detection of active nests, the site was first pre-baited by placing a tablespoon (approximately 20 g) of unpoisoned wallaby mince in each trap in order to attract wasps. Pre-baiting was performed in order to encourage large numbers of wasps to visit the traps before switching to the poisoned baits, as wasps returning to the nest carrying enticing food stimulate other wasps to seek out that food source (Archer 2012). After 1–3 days, the prebait was swapped with poisonous baits composed of wallaby mince mixed with 1 g/1000 g (0.1%) Fipronil. Wasps visiting the traps collected a small portion of poisoned meat and took it back to the nest to feed other workers, larvae and the queen who were then also killed, leading to the eventual demise of the nest. This process usually only took a few days after placing the poisonous baits. The baiting process was repeated at a site if wasp numbers increased again later in the season.

3.2.4 Data analysis

Minitab version 18.1 (Minitab Inc 2017) was used for inferential statistics. Multidimensional scaling and vector analysis were performed using DECODA (Database of Ecological Community Data, version 3) (Minchin 1989). The individual in the following analyses was observational transect by time.

Global non-metric multidimensional scaling was used to ordinate the transect segment floristic data following the default options in DECODA. Scores on a linear vector ($P < 0.001$) for butterfly abundance were calculated for each of the transects, producing a variable we call 'vegetation'.

A General Linear Model (GLM) using a logit-link function was built to determine whether vespid wasp numbers were affecting *O. ptunarra* butterfly numbers. The ranks of butterfly density (butterfly number/ha) as the response variable and wasp density (wasp number/ha) as a covariate, were used as the data were non-parametric. The two factors in the analysis, site and year, were treated as random variables to eliminate temporal and spatial autocorrelation.

Wasp transect data and wasp monitoring trap data were analysed to determine whether wasp control had been successful. A GLM was fitted to the wasp transect data using the rank of wasp density (number/ha) as the response variable, with year and wasp control performed (Y/N) as the factors. Year was again treated as a random variable to eliminate temporal autocorrelation. One-way ANOVA was performed on both the data sets to compare wasp density (wasp number/ha) to sites with and without wasp control in order to determine whether there was a change in wasp numbers. The percentage change was then calculated by comparing the means of the wasp density at sites where wasp control was present/absent.

A GLM was built to determine whether butterfly numbers had changed at wasp control sites. The rank of butterfly density (number/ha) was the response variable, with year and wasp control performed (Y/N) as the factors. Year was again treated as a random variable to eliminate temporal autocorrelation. One-way ANOVA was also performed on the rank of butterfly density (butterfly number/ ha) and sites where wasp control was present/absent and an interval plot was generated. The percentage of change was then calculated by comparing the absolute medians of the butterfly density at sites where wasp control was present/ absent.

Data for wasp and butterfly abundance from the period of 1998–2002 were compared to data from the current study (2011–2013) to determine whether wasp and butterfly density per hectare differed between the two time periods. Data for both time periods were only available for five sites (Dairymaid South Plain, Peak Plain, Racecourse Plain, South Hatfield Plain and Thompsons Plain). Data were pooled into 'old' and 'new' years and then analysed using one-way ANOVA.

The interaction between the environmental variables and both butterfly densities and wasp densities were analysed using multiple regression with best subsets to determine the best fitting models. The multiple regression analyses used either butterfly density ranked (number/ ha) or wasp density ranked (number/ha) as the response variable. The environmental variables determined to be the best predictors were used as the continuous predictors.

3.3 Results

Oreixenica ptunarra butterfly imagoes emerged in early March, peaking in numbers around the third week of March and then declining by early April (Fig. 3.2). Butterfly densities did not vary at the sites over the 3 years of the present study. However, the 2011–2013 butterfly numbers were 43.8% less than those for 1998–2002 (one-way ANOVA, $F = 4.25_{1, 150}$, $P = 0.041$). The environmental variables found to significantly affect butterfly density/ha (ranked) were plain area (ha) and time of day [regression: butterflies ranked/ha = 135.7 + 0.1206 plain area (ha) − 5.25 time AEST (decimal), $R^2 = 13.64\%$, P (plain area) = 0.003, P (time) = 0.008]. More butterflies were found on larger plains and butterflies decreased in numbers as the day progressed from 10 am onwards.

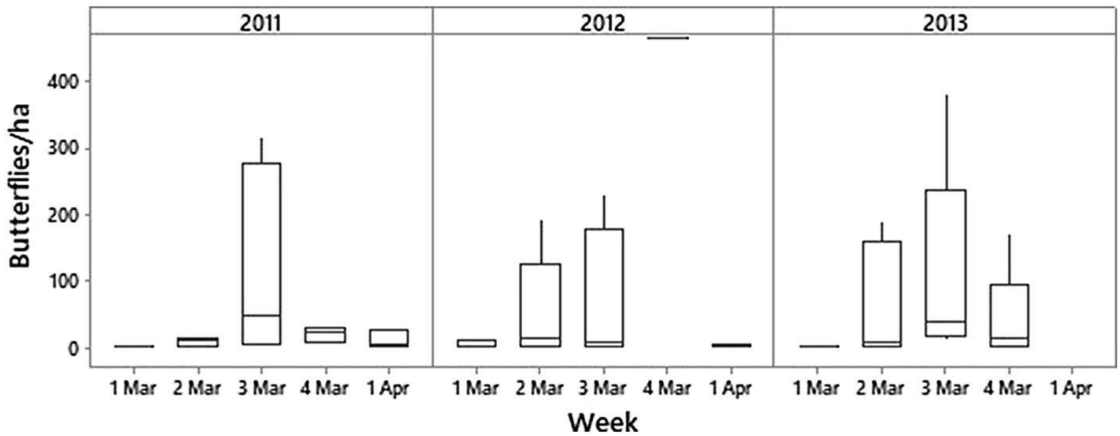


Fig. 3.2 Boxplot of butterfly densities at the study sites over 3 years 2011-2013

Vespid wasps started building their nests in spring, with wasp abundance increasing in March and peaking in early April when the new queens emerged from the nest and then declining in autumn when the nests generally perished due to cold weather. No overwintering nests were detected. Vespid wasp abundance increased at the study sites over the 3 years (one-way ANOVA, $F = 9.67_{2, 130}$, $P < 0.001$), with wasp numbers being low in 2011 and increasing in 2012 and 2013 respectively (Fig. 3.3). Wasp densities did not differ between 1998–2002 and 2011–2013. Both species of wasps were detected (*V. vulgaris* and *V. germanica*).

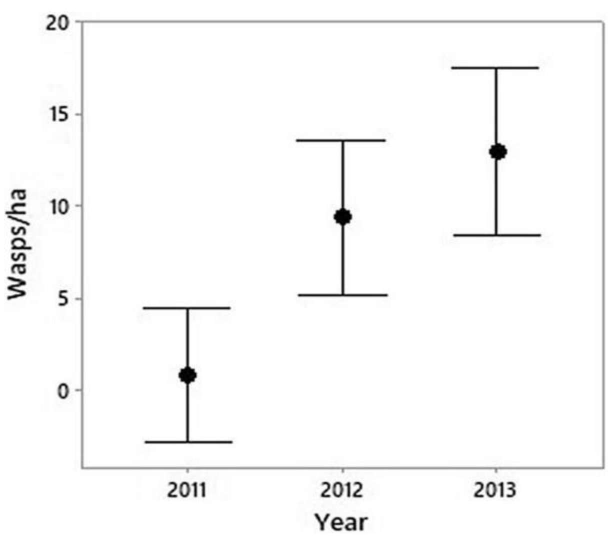


Fig. 3.3 Interval plot showing the wasp densities at the study sites over 3 years 2011–2013

The environmental variables found to significantly affect wasp density/ha (ranked) were temperature and vegetation [regression: wasps ranked/ha = 3.3 + 4.027 temperature (°C) + 27.25 vegetation, R^2 = 27.67%, *P* (temperature) ≤ 0.001, *P* (vegetation) ≤ 0.001]. Wasp numbers increased with increasing temperature and were found in greater numbers at the lower end of the vegetation vector, which indicated the degraded, overgrown sites.

The spring of 2010/11 had the highest percentage of days with potential ground frosts (< 2 °C) (Table 3.2). It also had the highest number of extended ground frosts (> 2 days), with 2011/12 and 2012/13 being increasingly warmer (Table 3.2). The spring rainfall for all of those years was below average, with 2010/11 only being marginally lower, while 2011/12 and 2012/13 were very dry years (Table 3.3).

Wasp control was found to have a significant effect on wasp density/ha (ranked) (GLM, Table 3.4). Both the transect data and the wasp monitoring trap data showed a significant decrease in wasp numbers at sites where wasp control was performed compared to non-control sites [transect data: oneway ANOVA, $F = 7.81_{1, 130}$, P = 0.006; wasp monitoring trap data: one-way ANOVA, $F = 6.05_{1,368}$, P = 0.014] (Fig. 3.4). Some outliers were present in 2013.

Table 3.2 Percentage of days with ground frosts and number of extended periods of frost in the spring (Sept–Nov) of 2010–2012

Date	Days < 2 °C (%)	Extended frosts (>2 days)
2010/11	42	6
2011/12	32	4
2012/13	29	3

Table 3.3 Total spring rainfall (Sept–Nov) and the the differences between the actual and the average spring rainfall for 2010–2012

Year	Total spring rainfall	Difference from average rainfall
2010/11	552.9	− 30.2
2011/12	319.1	− 264.0
2012/13	276.8	− 306.3

Table 3.4 Attributes of general linear model showing that wasp control was found to have a significant effect on wasp density

Source	DF	Adj SS	Adj MS	F	*P*
Year	2	53,051	26525.3	26.78	0.000
Wasp control (Y/N)	1	16,279	16278.9	16.44	0.000
Error	127	125,780	990.4		
Lack-of-fit	2	12,061	6030.3	6.63	0.002
Pure error	125	113,719	909.8		
Total	130	192,958			

Wasp density/ha ranked is the response variable, with wasp control performed (Y/N) as a fixed factor and year as a random factor
R^2 = 34.81%

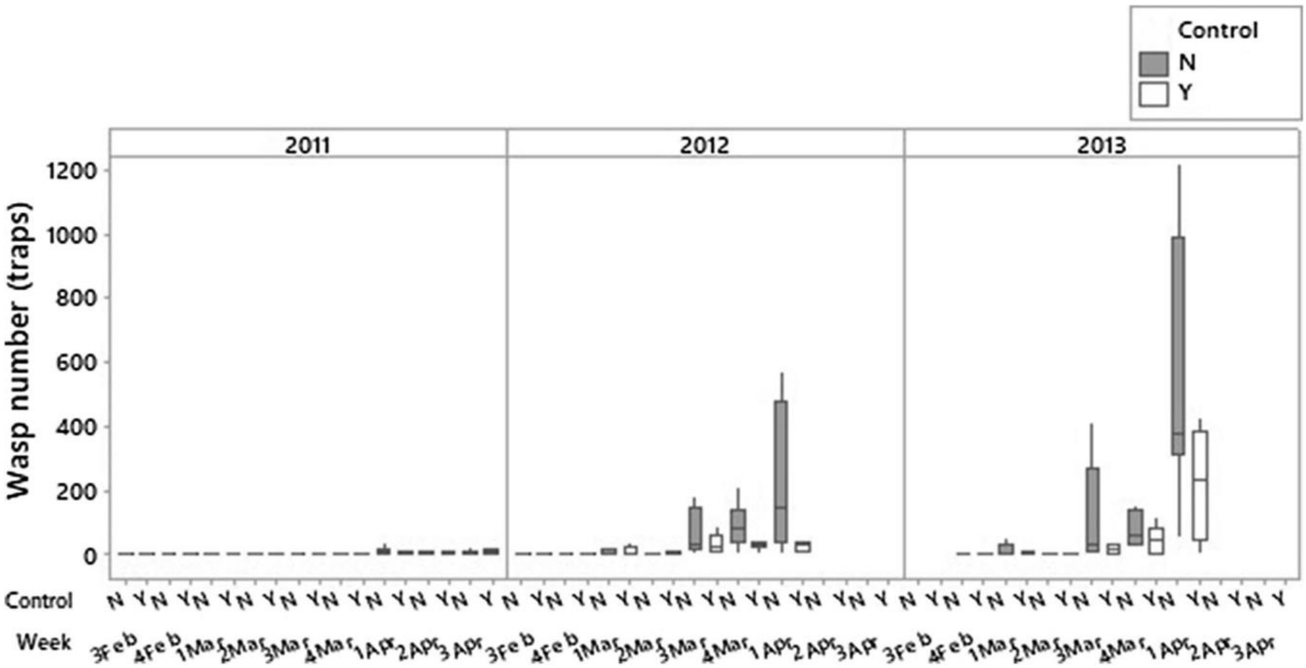
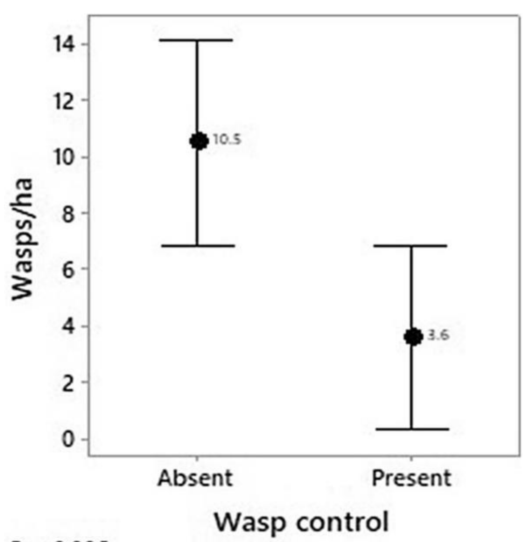


Fig. 3.4 Boxplot showing wasp densities at sites where wasp control was present or absent, from late February to early April 2011, 2012 and 2013, from wasp monitoring trap data

The transect data showed that wasp control was effective at reducing vespid wasp abundance by almost two-thirds (65.7%) of their number compared to untreated sites (Fig. 3.5).

Vespid wasp numbers were found to significantly affect *O. ptunarra* butterfly numbers when the effects of site and year were randomised (GLM, Table 3.5), with sites with fewer wasps supporting higher numbers of butterflies. Furthermore, wasp control was found to have a significant effect on butterfly density/ha (ranked) (GLM, Table 3.6), with the transect data showing that *O. ptunarra* butterfly numbers (ranked) were 10.1% higher at wasp control sites than untreated sites (one-way ANOVA, $F = 4.99_{1,130}$, $P = 0.027$) (Fig. 3.6).



P = 0.006

Fig. 3.5 Interval plot showing the wasp densities at sites where wasp control occurred and sites where it was absent, from transect data

Table 3.5 Attributes of general linear model showing that wasp numbers significantly affect butterfly numbers

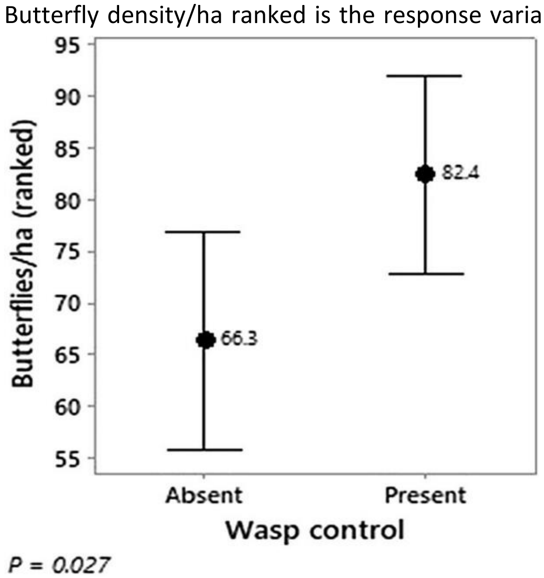
Source	DF	Adj SS	Adj MS	F	*P*
Wasp density/ha (ranked)	1	8226	8225.8	7.67	0.006
Plain area (ha)	8	97,443	12180.4	11.36	0.000
Year	2	6775	3387.7	3.16	0.046
Error	119	127,542	1071.8		
Lack-of-fit	54	38,167	706.8	0.51	0.994
Pure error	65	89,375	1375.0		
Total	130	226,881			

Butterfly density/ha ranked is the response variable, wasp density/ha ranked is the covariate, with plain and year as random factors
R^2 = 43.78%

Table 3.6 Attributes of general linear model showing that wasp control was found to have a significant effect on butterfly density

Source	DF	Adj SS	Adj MS	F	*P*
Year	2	1036	517.8	0.30	0.739
Wasp control (Y/N)	1	8609	8609.0	5.03	0.027
Error	127	217,399	1711.8		
Lack-of-fit	2	2665	1332.4	0.78	0.463
Pure error	125	214,734	1717.9		
Total	130	226,881			

Butterfly density/ha ranked is the response variable, with wasp control performed (Y/N) as a fixed factor and year as a



random factor
R^2 = 4.18%

Fig. 3.6 Interval plot showing the butterfly densities (ranked) at sites where wasp control occurred and sites where it was absent, from transect data

3.4 Discussion

It is doubtful that the increase in abundance of wasps between 2011 and 2013 is part of an ongoing rise in wasp numbers, as vespid wasps have been present in the area for many years. After an initial increase, numbers of invasive species tend to reach a plateau. Elsewhere, wasp populations also fluctuate (Lester et al. 2017; Spradbery 1973b).

It is possible that some of the variation could be explained by differential effects between the two *Vespula* species, which could be investigated further. The two species' distribution varies slightly in Tasmania with *V. germanica* preferring open or partially shaded sites, while *V. vulgaris* can forage and build nests in closed canopy forests adjacent to open sites (Bashford 2010) and they may still be in a process of determining this balance. Ultimately, the two species utilise the same resources and will impact *O. ptunarra* numbers no matter which is present.

It is likely that most of the fluctuations in wasp numbers are largely due to variation in spring weather conditions (Masciocchi et al. 2016), and competition between queens for nest sites in areas of high wasp abundance (Barlow et al. 2002; Lester et al. 2017). Competition between queens is difficult to quantify and has not been measured in the present study but may have had some effect in addition to weather.

Spring is when the recently emerged queens are starting to build their nests and are at their most vulnerable, as they do not yet have workers to feed them, assist with the nest building and to keep the nest warm (Masciocchi et al. 2016). Excessive rain in spring can flood nests and ground frosts can chill them, either of which can kill the queen (Madden 1981). The combination of cool spring temperatures and high rainfall leads to less nest success and, consequently, a lower abundance of wasps that season; while warmer, drier conditions lead to years of high abundance (Masciocchi et al. 2016).

Although the carrying capacity of a site is largely determined by climatic conditions and competition between queens (Barlow et al. 2002), there may also be competition for resources between established wasp nests. A previous study in Tasmania determined that the availability of insect prey influenced the abundance of wasp nests (Madden 1981). In metropolitan Sydney, where availability of food was not a limiting factor, it was determined that rainwater for drinking and the formation of wood pulp affected the abundance of wasp nests (Horwood et al. 1993). While the density of vespid wasp nests has not been recorded in Australia it has been found to be as high as 30 nests per ha in the New Zealand *Nothofagus* beech forests (Barlow et al. 2002), while it is much lower at 0.1 to 1.7 nests per hectare in their native range in the UK (Archer 2001).

Wasp abundance decreased significantly in low temperatures in the present study. Furthermore, the wet, cold conditions in the spring of 2010/11 may have resulted in the death of many vulnerable, recently emerged queens, resulting in the lower abundance of wasps that year. The increasingly drier and warmer spring conditions of 2011/12 and 2012/13 then may have resulted in the increased abundance of wasps as more young queens survived and more nests were built. Drier, warmer years may become more prevalent with global warming, increasing the number of years of high wasp abundance (Lester et al. 2017). The high outliers in Fig. 3.4 in 2013 were most probably due to a boom in wasp numbers at a few of the sites, due to the emergence of the new queens in autumn. While the low outliers were at sites that experienced lower wasp numbers overall and where the new queens had not yet started emerging.

Wasp control was successful in reducing vespid wasp abundance by 65.7%, with a subsequent rise in butterfly numbers at these sites, indicating that vespid wasps are having a negative impact on numbers of *O. ptunarra*, and that it is possible to reduce the impact of these predators on the butterfly. The increase in *O. ptunarra* numbers was small, indicating that wasp control effort may need to be increased.

Reducing vespid wasp numbers by 55–70% (Beggs and Rees 1999) and 59.1% (Toft and Rees 1998) was not sufficient to protect free-living caterpillars or orb-web spiders respectively in beech forests on the South Island of New Zealand. Beggs and Rees (1999) deduced that vespid wasp numbers need to be reduced by 85–91% of their original numbers, which equates to about 2.7 wasps per malaise trap per day, in order to adequately protect invertebrates; which is supported by Toft and Rees (1998) who required a reduction of 80–95% or around 5.5 wasps per malaise trap per day. In order to adequately protect *O. ptunarra*, it is likely that wasp control efforts will need to be increased, which often has major cost implications.

Currently wasp traps were placed in a ring around the perimeter of the grassland spaced 300 m apart at a density of 0.1–1.3 traps per hectare under the assumption that all nearby wasp nests would be exterminated, as wasps generally do not fly further than 400 m from the nest while foraging (Archer 2012). It was thought that the line of traps would act as a barrier to wasps entering the grasslands from adjacent vegetation by luring them to the poisoned baits and consequently killing nearby nests. However, this trap density is low compared to other studies [i.e. 7.7–8 traps/ha (Beggs and Rees 1999) and (Toft and Rees 1998), 2.2/ha and 4.98/ha (Harris and Etheridge 2001) and 13.3/ha (Sackmann et al. 2001)], which may explain the relatively low wasp reduction rate.

Ways to increase the wasp control effort include placing a higher density of traps around the perimeter of the grasslands, or placing the traps in a high-density grid pattern within the grasslands, or by clustering bait stations together at intervals within the grasslands. Baiting in a systematic grid is a favoured method as it is likely to increase the chances of foragers from most nests encountering the bait, as opposed to baiting along a straight line which had variable results (Harris and Etheridge 2001). Both of these methods would entail an increase in labour intensity and thus more costs, particularly the grid method as many more traps would be involved (Beggs et al. 1998). A recent study found that clustered bait stations were able to kill a large number of wasps in a short time with less effort required than intensive grid baiting systems (Harper et al. 2015). They recorded an 80% reduction in wasp traffic at nests within 113 m of a cluster and a 50% reduction at nests within 250 m, with no reduction at 470 m. This may be an effective technique to trial at the Surrey Hills grasslands for a greater reduction in wasp numbers with only a minimal increase in effort.

Changing the timing of the wasp eradication is unlikely to increase its efficacy. Wasp eradication was performed when it is most effective during the wasps' peak time of Feb–Mar. Poisoning is not as effective in earlier months during Spring, as not enough foraging wasps visit the baited traps (Beggs et al. 1998). Similarly, poisoning in later months during Autumn is not ideal either as the queens will have dispersed and wasp numbers are waning, indicating that not much ecological gain would be achieved, as poisoning would not greatly reduce numbers in time for next season (Beggs et al. 1998).

Different toxins or bait could potentially be used to increase the efficiency of the wasp control. However, fipronil was chosen as it has been shown to be the most effective wasp poison currently available (Harper et al. 2015) (Harris and Etheridge 2001) and wallaby mince was selected as it has previously been found to attract *Vespula* spp. in Tasmania (Bashford 2010). It could prove beneficial to add an attractant such as heptyl butyrate to the baits to increase the rate of wasp visitation, which was found to be an effective attractant for *V. germanica* (Buteler et al. 2018) and significantly increased visitation by *V. pensylvanica* (Saussure) to fipronil baits in Hawaii (Hanna et al. 2012).

A potential alternate toxin that has not yet been used extensively for vespid wasps is the insect growth regulator fenoxycarb, which kills the larvae and also inhibits egg production by the queen but does not directly affect the workers, enabling them to continue collecting poisoned bait and delivering it to the nest (Banks et al. 1988). Fenoxycarb has been widely used for controlling other insects such as imported red fire ants (*Solenopsis invicta* Buren, 1972) (Banks et al. 1988) and agricultural pests such as diamond back moths (*Plutella xylostella* Linnaeus, 1758) in Iran (Mahmoudvand and Moharramipour 2015) and is currently being considered for controlling the Asian hornet (*Vespa velutina nigrithorax* Lepeletier, 1836) in France (Turchi and Derijard 2018).

Biological control is another potential method for controlling vespid wasps, however currently no effective parasitoids have been identified. The promising parasitoid *Sphecophaga vesparum vesparum* Curtis, 1828, was released in New Zealand where it did not significantly reduce vespid wasp numbers, even when parasitoid density was high (Beggs et al. 2008). Some initial trials were also performed in Australia with this parasitoid (Field and Darby 1991), which were not successful (Beggs et al. 2011). Later releases of the parasitoids *S. vesparum burra* Cresson, 1869 and *S. orientalis* Donovan, 2003 in New Zealand were also unsuccessful (Beggs et al. 2002; Donovan et al. 2002).

The decrease in abundance of *O. ptunarra* butterflies between 1998–2002 and 2011–2013 may have occurred when the introduced wasps first appeared in the area. As vespid wasps are generalist predators that switch to other prey species when the abundance of a prey becomes low, some prey species are able to persist at low densities without becoming extinct (Beggs 2001). The abundance of low-density prey species should rise again if wasps are removed from the community or if their numbers are sufficiently reduced (Sackmann et al. 2008). *O. ptunarra* numbers appear to have been kept at low densities due to vespid wasp predation, and when predation pressure was lifted due to wasp control efforts, butterfly abundance rose. In areas of high wasp densities, high levels of predation can erode genetic diversity, leading to a genetic bottleneck (Burne et al. 2017). Additionally, if densities of butterflies further decrease, local extinctions may occur. Thus, it would be beneficial for the ongoing survival of *O. ptunarra* if wasp control efforts were increased and performed at key sites across its range.

As well as increasing the wasp control efforts at key sites, other conservation management methods should also be applied to protect *O. ptunarra*. It would be beneficial to place buffers around the edges of the grasslands to exclude disturbance such as roading and forest plantations, as disturbed ground is favoured by wasps for nest building. Managing grasslands to keep them from becoming overgrown would also be beneficial, as significantly more wasps were present in the degraded, overgrown sites. As there are significantly higher densities of *O. ptunarra* within larger grasslands, these are key sites for conservation. Also, translocating *O. ptunarra* to viable sites within its historic range in order to create new self-sustaining populations would also assist in expanding the butterflies' range and protect them from extinction caused by stochastic events.

3.5 Conclusion

Controlling vespid wasps through the use of poisons was successful in significantly reducing wasp numbers and led to a small increase in *O. ptunarra* numbers. Although butterfly numbers have decreased over the last 15 years, the abundance of *O. ptunarra* stayed relatively stable at the sites over the study period. It is believed that wasps are keeping the butterflies at low densities and in order to recover populations of *O. ptunarra* as well as provide ongoing protection, the wasp control effort needs to be increased in conjunction with other forms of conservation management such as buffers around grasslands and translocating the species to other viable sites. Without ongoing conservation measures, it is likely that butterfly numbers will stay low, potentially leading to genetic bottlenecks and local extinctions.

Chapter 4 A successful translocation of the threatened ptunarra brown butterfly (Oreixenica ptunarra) in the highland Poa grasslands of Tasmania, Australia

Abstract

Translocation has been used as a conservation tool in Tasmania, Australia to assist in the recovery of the threatened ptunarra brown butterfly (*Oreixenica ptunarra*) by attempting to establish new populations within its historical range. *O. ptunarra* is endemic to Tasmania, where its numbers have declined in recent years, with localised extinctions of populations occurring at some sites. Translocations were trialled to counteract these local extinctions by establishing new, viable, self-sustaining populations within the species' historical range. Gravid female butterflies and eggs were translocated to four experimental sites over a four-year period, with a new population being successfully established at one site. The successful translocation site was associated with a high flower richness, suggesting that nectar is an essential attribute for future translocation sites.

4.1 Introduction

The number of threatened species is rising worldwide due to the loss and fragmentation of habitat, overexploitation, increases in predation and competition by introduced species, and more recently by climate change (Hoffmann et al. 2010). When a species' habitat becomes unsuitable because of such threats, it will often migrate to other areas of suitable habitat where these pressures are reduced or absent. However, some species are unable to migrate due to factors such as limited dispersal abilities, lack of connecting habitat or low population growth rates, and, in these cases, human intervention through translocation may become necessary (Carroll et al. 2009). There has been a large increase in the number of translocations performed since this method has been recognised as a useful conservation tool (Seddon 2010).

A successful translocation results in a wild, self-sustaining population, which requires minimal ongoing management (IUCN 1998). Two main types of translocation are recognised: moving species within their historical range to places where they are no longer extant, and moving species to places where there is no historical record of their occurrence (Seddon 2010). Conventional guidelines on translocations typically endorse the former type, provided that the threats leading to the original extinction have been removed (IUCN/SSC 2013) and that care has been taken that population size and genetic diversity are maintained (Schmitt et al. 2005). Translocations outside of the historical range have typically been met with disapproval because of the risks to both the species and the receptor ecosystem. Such translocations can prove to be harmful to the environment if not thoroughly researched and planned prior to the movement, because the introduced species may perform in unexpected ways which may have adverse consequences on the ecosystem (Davidson and Simkanin 2008). However, the reluctance to translocate outside known ranges is reducing because climate change can mean that the only suitable habitat that is available is now outside historical ranges (Seddon 2010).

Prime examples of species that have had adverse consequences on ecosystems are exotic species such as weeds, feral animals and biological control agents which have been accidentally or purposefully introduced into an ecosystem in which they do not naturally occur, and have thrived, often to the detriment of native species (IUCN 1987; Simberloff 2005). A case in point is the introduction of the cane toad (*Bufo marinus* L.) into Australia in the 1930s as a biological control agent for cane beetles (Scarabaeidae), a damaging pest of sugarcane crops. The toads established and spread quickly and now occupy most of the tropics and subtropics of Australia.

They cause severe environmental impacts, as they are toxic to predators, eat a wide variety of native invertebrates and small vertebrates and are a vector for at least two diseases (Shanmuganathan et al. 2010).

The conservation management of species through translocations should be performed with caution and used only as an interim tool, while other conservation actions are being developed. Although it may be relatively easy to move individuals to different locations, the process of translocation does not address the underlying threats to the species such as habitat loss, predation or chemical use, which must be rectified to effectively conserve the species. Translocations should only be used in urgent situations in the short-term, while other management strategies are being developed.

Butterflies are a group for which conservation translocations have been recently attempted in various locations, as butterflies are valued by the public and all life stages can be easily and relatively inexpensively moved from one area to another (Schultz et al. 2008). Monitoring translocation success is also straightforward using well established protocols such as the use of transects, quadrats or mark-capture-release methods, as most species do not move far from their host plants.

In the UK and the USA, translocations of at-risk butterfly species are a common part of the recovery or action plans for these species (Schultz et al. 2008). Translocation methods vary between countries, with the UK favouring the release of adult butterflies, while the USA also utilises larvae and pupae (Schultz et al. 2008). Only a fraction of translocations are successful. A past review having determined that, out of 226 attempts for 25 butterfly species in the UK and USA, only 29 translocations were successful (Schultz et al. 2008), as the requirements of the butterflies were often not thoroughly researched (Oates and Warren 1990). Most successful translocations have been into areas with suitable habitat and climate that are large enough in size to support a butterfly population (Oates and Warren 1990).

In the UK, translocations of the heath fritillary butterfly (*Melitaea athalia*) and the large blue butterfly (*Phengaris arion*) were attempted, as their numbers had declined due to lack of suitable habitat. Both were successfully translocated into habitats that had been restored after becoming overgrown due to changes in land use (Pullin 1996). *P. arion* additionally required the correct species of obligate ant (*Myrmica sabuleti* Meinert, 1861) to be present before the translocation was successful. Both species thrived after being moved to the restored areas (Pullin 1996).

In the UK, translocations of the large copper butterfly (*Lycaena dispar* (Haworth, 1802)) were attempted, as this species had become extinct in the UK due to the loss of its wetland habitat but had persisted in mainland Europe. A translocation of *L. dispar* from Europe back to the UK was attempted but proved to be problematic. Initially a bivoltine subspecies of butterfly from central Europe was used which was not suited to the climate and the translocation failed. Next, translocation of a univoltine subspecies from the Netherlands was successful but a larger area than was available would have been needed for the population to remain viable in the long-term, as the species required a network of sites for its dispersal strategy (Pullin 1996; Pullin 1997).

In Australia, examples of butterfly translocations include one undertaken to protect a threatened species in an emergency and others intended to increase the range of declining species. The emergency translocation was to protect the threatened purple copper butterfly (*Paralucia spinifera*), which was detected during roadworks near Lidsdale, New South Wales, and was successfully performed by moving 1260 caterpillars from plants earmarked for removal in the course of the roadworks to an area of adjacent habitat that also contained the host ant species (Mjadwesch and Nally 2008).

Translocations were also performed in Melbourne, Victoria to increase the range of two butterfly species whose habitat had diminished due to urban encroachment. The splendid ochre butterfly (*Trapezites symmomus*

Hübner, 1823) was successfully translocated to the Gresswell Forest Nature Conservation Reserve by moving 172 larvae and some shelters, after first increasing the size and quality of the habitat (Braby 2012). From here the species colonised the nearby La Trobe Wildlife Reserve which also contained suitable habitat. However, the attempt to translocate six larvae of the sword-grass brown butterfly (*Tisiphone abeona albifascia* Waterhouse, 1904) into swamp forests containing the host plant *Gahnia sieberiana* Kunth in eastern Melbourne was unsuccessful, possibly because too few individuals were moved (Belvedere et al. 1998).

The threatened ptunarra brown butterfly (*Oreixenica ptunarra*) is a small orange-brown butterfly endemic to highland *Poa* grasslands of Tasmania, Australia. *O. ptunarra* is listed as vulnerable and endangered under State and Federal government legislation, respectively, primarily due to habitat loss through land clearing, fragmentation of habitat, unsuitable fire and grazing regimes (Threatened Species Unit 1998) and, more recently, predation by introduced vespid wasps (*Vespula germanica and V. vulgaris*) (Potter-Craven et al. 2018). Climate change is another recent threat to *O. ptunarra* which has not yet been quantified or included in recovery plans. The climate in western Tasmania has changed over the period 1979-2016, with an increase in rainfall variability, resulting in more extreme dry and wet periods; as well as an increase in the incidence of dry lightning promoting more wildfires (Styger et al. 2018). It is possible that this change may affect the habitat of *O. ptunarra* through a gradual change in vegetation composition in the *Poa* grasslands.

A long-term monitoring program in the *Poa* highland grasslands of northwestern Tasmania observed a dramatic decline in *O. ptunarra* numbers (Potter-Craven et al. 2018), with cases of localised extinctions of some populations. The butterflies have been unable to recolonise these areas because they are not able to fly the long distances between the fragmented grasslands through unsuitable habitat. *O. ptunarra* would benefit from translocation, which would facilitate the expansion of the species into suitable habitat within its historical range and increase the number of viable populations. Although translocation should be used with caution, it was deemed necessary in this case, due to the ongoing threat of vespid wasp predation and the lack of corridors through which the butterflies could recolonise sites where *O. ptunarra* has become extirpated. Translocation was also identified in the most recent recovery plan for the species (Bell 1999) as an action required to ensure the long-term persistence of *O. ptunarra*.

The preferred host plant for the larvae of *O. ptunarra* is *Poa labillardierei,* but they also occur on other *Poa* species, with the eggs being laid directly into grass tussocks (Neyland 1993). The caterpillars live and feed upon the *Poa* until they pupate within the tussock. When the imagoes emerge, they are generalist nectar feeders that visit an assortment of flowers in bloom on the grasslands, and around their margins, during their brief flying period in mid-autumn (Anderson 2001).

O. ptunarra was once widespread across central Tasmania in grassland, grassy shrubland and open, grassy woodland habitats containing *Poa* (Threatened Species Unit 1998). However, there has been a large decline in habitat for the species since European settlement largely due to land clearing. Around 40% of the original area of native grassland in Tasmania was lost in the period 1802-1995, the majority of which was *Poa* grassland (Kirkpatrick et al. 1995). Due to this loss and the ongoing decline of the grasslands, the highland *Poa* grasslands are now listed as a threatened native vegetation community under Tasmanian State government legislation, while the lowland native grasslands of Tasmania are listed as critically endangered under Australian federal government legislation.

The aims of the present paper are to determine the prerequisites for the successful translocation of *O. ptunarra* including: the vegetation composition requirements of sites containing *O. ptunarra*, the number of individuals and/or eggs that needed to be moved to ensure establishment, and a suitable method of butterfly collection and release.

4.2 Methods

4.2.1 Translocation

The present study was conducted at fifteen sites in northwestern Tasmania, Australia, near the town of Waratah (41.446°S 145.532°E, 600 m asl) (Fig. 4.1). The sites comprised of: one translocation source site, four translocation release sites, four wasp monitoring sites and six additional vegetation survey sites (Table 4.1). The sites occurred within the privately-owned Surrey Hills area and were interspersed with forestry plantations, remnants of native forest vegetation and clear-felled ground. The vegetation at the sites was classified as 'highland *Poa labillardierei* grassland' which is a preferred host plant of the larvae of *O. ptunarra* (Harris and Kitchener 2005b). Flowering dicotyledonous plants, whose nectar is consumed by the butterflies, were also present in varying abundance.

Populations of *O. ptunarra* were present at all of the sites except for one of the vegetation survey sites (Muddy Creek Plain) and the four translocation release sites. Predatory introduced vespid wasps were present at all sites (Table 4.1).

The study area has a cool temperate climate and receives an average of 2000–2400 mm rainfall/year, with no month receiving <100 mm (Bureau of Meteorology 2011). The underlying geology of all the sites was Tertiary basalt (Land Information Systems Tasmania 2014). Native vertebrate herbivores, mainly wallabies and wombats, grazed all the sites.

Translocations of *O. ptunarra* were performed annually from 2009 to 2012 during the normal flight period in late March. The translocations in 2009 and 2010 were performed by the Threatened Species Section of the Tasmanian Department of Primary Industries, Parks, Water and Environment (DPIPWE) together with private land managers and the author. The study was then taken over by the author, with further translocations performed in 2011 and 2012.

This research complied with the DPIPWE Translocation Policy and was performed under the authority of Threatened Fauna Permit numbers TFA 11061, TFA 12060 and staff permits. It was determined that this research was unlikely to have a detrimental impact on the source population and was warranted by the potential for creating new populations of *O. ptunarra*.

Before translocating *O. ptunarra*, a qualitative assessment was made of potential release sites to be used. Sites were typically selected for their small, easy to manage size and the abundance of *Poa* habitat plants present. The presence of flowering nectar sources was considered a bonus but was not thought to be essential since relatively few grassland plants flower in mid-autumn.

Translocations were performed by moving *O. ptunarra* from the source site to four suitable release sites within the species' historical range. The source site (South Hatfield Plain) had a large self-sustaining population of thousands of *O. ptunarra* over > 120 hectares and is one of the best sites in Tasmania for the butterflies. The four release sites (Hatfield Valley Plain, Hummocks Road Plain, Thompsons Park Plain and Dairymaid North Plain) were all small enough to be easily managed and defended against predatory vespid wasps, contained suitable *Poa* habitat, occurred within the species' historical range and presently lacked *O. ptunarra.* The four wasp monitoring sites (Twyford Creek Plain, Guildford Plain, Westwing Plain and Racecourse Plain), were surveyed weekly to compare vespid wasp numbers at these sites with the translocation release sites and to determine whether wasp control had been effective at the release sites. Vegetation surveys were performed at all of the aforementioned sites, as well as an additional six vegetation survey sites (Dairymaid South Plain, Huskisson Plain, Muddy Creek Plain, Moory Mount Plain, Vale of Belvoir and Peak Plain) (Fig. 4.1 and Table 4.1).

Table 4.1 Details of the study sites including size, site treatment, butterfly status, trap numbers and wasp control trap density

Plain	Size (ha)	Site Treatment	Butterfly Status (Present/ Absent/ Extinct)	No. of monitoring traps	No. of wasp control traps	Density of wasp control traps / ha
South Hatfield	128.3	Translocation Source	Present	2	14	0.1
Dairymaid North	4.5	Translocation release, wasp control & vegetation surveys	Absent	1	4	0.9
Hatfield Valley	6.3	Translocation release, wasp control & vegetation surveys	Absent	1	6	1.0
Hummocks Road	6.0	Translocation release, wasp control & vegetation surveys	Absent	1	3	0.5
Thompsons Park	32.0	Translocation release, wasp control & vegetation surveys	Extinct	3	8	0.3
Guildford	75.7	Wasp monitoring & vegetation surveys	Present	2	-	-
Twyford Creek	72.3	Wasp monitoring & vegetation surveys	Present	2	-	-
Racecourse	324.0	Wasp monitoring & vegetation surveys	Present	3	-	-
Westwing	94.9	Wasp monitoring & vegetation surveys	Present	4	-	-
Dairymaid South	22.4	Vegetation surveys	Present	n/a	n/a	n/a
Huskisson	34.3	Vegetation surveys	Present	n/a	n/a	n/a
Moory Mount	4.0	Vegetation surveys	Present	n/a	n/a	n/a
Muddy Creek	5.0	Vegetation surveys	Absent	n/a	n/a	n/a
Peak	38.7	Vegetation surveys	Present	n/a	n/a	n/a
Vale of Belvoir	963.2	Vegetation surveys	Present	n/a	n/a	n/a

The release sites were initially surveyed for *O. ptunarra* to establish their suitability as translocation sites, since the translocations were to be performed to sites within the historical range of *O. ptunarra* but which did not currently contain the species. Following this, surveys for *O. ptunarra* were performed at the release sites both before and after translocation to determine if the species was present. Butterfly surveys were performed prior to the translocations as a precautionary measure, as the initial surveys had determined that *O. ptunarra* were absent from these sites. Butterfly surveys were performed after the translocations were carried out to determine whether the translocations had been successful. If *O. ptunarra* were detected following a translocation, then this indicated that the translocation had been successful, and no further translocations were performed at this site. If *O. ptunarra* were not detected, then it was assumed that the translocation attempts had been unsuccessful.

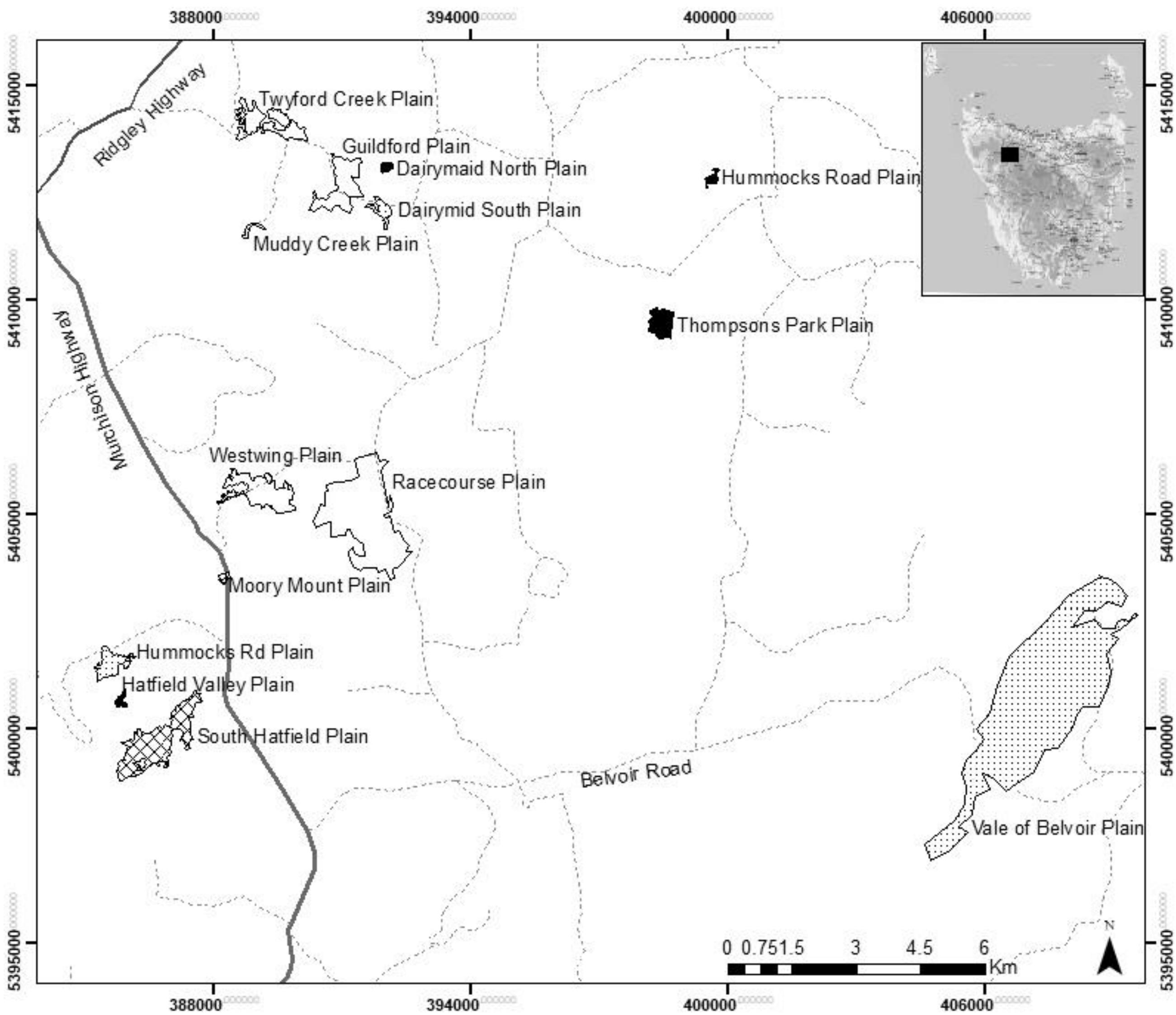


Fig. 4.1 Location of the fifteen sites showing treatment: translocation source site (hatched), translocation release sites (black), wasp monitoring sites (white), additional vegetation survey sites (dotted)

The release sites were regularly monitored for the presence of vespid wasps and *O. ptunarra.* Translocations were only performed to sites where vespid wasp numbers were low, as otherwise it was likely that the translocated butterflies would be killed by wasp predation. Translocations were also not performed to sites that contained *O. ptunarra*, as their presence was an indication that a previous translocation performed during this study had been successful.

A previous review of translocation studies determined that there was no correlation between the release of high numbers of individuals (>50 adults or >250 larvae) and success. Successes often had moderate numbers of adults (30-50) or larvae (<250) (insert Oates and Warren, 1990). In light of this, and following discussions with *O. ptunarra* experts, it was determined to translocate 50 female imagoes to each receptor site per year, as well as collected eggs. Larvae were not used, as they are difficult to capture and identify in the field. In 2009/2010 only 150 female butterflies were approved to be translocated, with up to 50 imagoes permitted at each site, as well as any eggs laid en route. This was problematic, as unequal numbers of butterflies were being released at the sites, with some sites receiving fewer than 50 butterflies. The 2011/2012 permits allowed for 50 female butterflies to be translocated to each of the four release sites per year, as well as the eggs from another 100 females, annually for three years (Table 4.2). Only female imagoes were translocated, as it was assumed that the majority of the females would have been fertilised, since mating occurs shortly after emergence and unmated females were very scarce in the field (Anderson 2001). The butterflies were collected from a different area within the source site each year to disperse the impact of the butterfly and egg removal. Translocations did not always proceed at all of the release sites due to the presence of vespid wasps.

The translocated imagoes were caught at the source site using butterfly nets and placed within individual clear plastic vials within a cool, dark, insulated cooler. The butterflies were transported to the release sites on the same day within a few hours of capture to maximise their survival. Translocated butterflies were offered a tissue wick infused with a 10% sugar solution and placed in the sun to feed and warm them before release. Imago translocations occurred annually from 2009 to 2012 (Table 4.2).

The 100 female butterflies that were caught for egg collection were placed in clear, plastic vials and kept in a shaded location for a maximum of four and a half hours to encourage them to lay eggs. The butterflies were then offered a tissue wick infused with a 10% sugar solution and re-released at the source site at the approximate location where they were caught. The eggs laid inside the plastic vials were translocated to the same release sites as the imagoes on the day of collection. Each egg was gently removed from the vial with a clean paintbrush or finger and dropped into the centre of a separate *Poa* tussock. The additional translocation of butterfly eggs was performed annually during the period 2011-2012 (Table 4.2).

In 2009 translocation of imagoes occurred at all the release sites, as vespid wasp numbers were low at all sites. Fifty butterflies were translocated to two sites (although one died en route) and 25 butterflies were translocated to the other two sites. The 25 eggs that were laid en route were translocated to the same sites as the imagoes. In 2010 and 2011 imago and egg translocations occurred only at Hatfield Valley Plain, and in 2012 translocations occurred only at Thompsons Park Plain and Hummocks Road Plain, as these were the only release sites with sufficiently low wasp numbers that were devoid of *O. ptunarra*.

Table 4.2 Numbers of female *O. ptunarra* butterflies and eggs translocated to release sites during 2009-2012

Release site	Number of female butterflies translocated				Number of eggs translocated			
	2009 20-25 Mar	2010 25 Mar	2011 26-27 Mar	2012 27-29 Mar	2009 20-25 Mar	2010 25 Mar	2011 26-27 Mar	2012 27-29 Mar
Hatfield Valley	25	50	50	0	2	91	76[2]	0
Hummocks Road	50	0	0	50	20	-	0	4
Thompsons Park	49[1]	0	0	50	3	-	0	12[1]
Dairymaid North	25	0	0	0	-	-	0	0

[1] One butterfly fatality. [2] Two butterfly fatalities.

The egg collection yielded low numbers in 2012, with only 12 eggs being collected from 100 females, compared to 76 eggs collected in 2011 and 91 eggs laid en route in 2010. Therefore, in 2012 it was determined to put all the eggs at a single release site (Thompsons Park Plain). However, the four eggs laid en route by the female butterflies being translocated to Hummocks Road Plain were placed there.

The translocation and egg collection occurred several days later in March each year from 2009 to 2012 due to time constraints. By 2012 the translocation and egg collection occurred approximately a week later (27-29 March) compared to the translocation in 2009 (20-25 March).

4.2.2 Monitoring

Butterfly and vespid wasp numbers were monitored at the release sites and at wasp monitoring sites (Fig. 4.1) by counting individuals along a marked transect at approximately weekly intervals from early March to early April 2009-2013, as described in Potter-Craven et al. (2018). Wasp populations were further monitored by also counting the number of wasps caught in monitoring traps at approximately weekly intervals at each site. Vespid wasps were monitored to determine whether wasp numbers were high, in which case *O. ptunarra* was not translocated, as the wasps would predate upon the butterflies.

The release sites were initially surveyed for *O. ptunarra* to establish their suitability as translocation sites and to ensure that O. ptunarra was not present. Following this, surveys for *O. ptunarra* were performed at the release sites before the translocations as a precautionary measure to verify that the species was still not present. Butterfly surveys were also performed after translocations were carried out to determine whether the translocations had been successful. If *O. ptunarra* were detected, then this indicated that the translocation had been successful and no further translocations were performed at this site. If *O. ptunarra* were not detected, then it was assumed that the translocation attempts had been unsuccessful.

4.2.3 Wasp control

Vespid wasp control was performed as described in Potter-Craven et al. (2018) from mid-February to early April in 2011-2013, when wasp numbers were elevated. Wasp control was performed for an additional year after translocations ended to give small *O. ptunarra* populations a better chance of establishment and survival. Any wasp control carried out prior to 2011 was performed at a very low level and although the effectiveness was not measured, it would likely have had a negligible impact on vespid wasp numbers. Butterflies were only moved to a translocation site if the wasp numbers were low at that site. A site with low wasp numbers was defined as one that had <60 wasps collected in an onsite wasp monitoring trap over an interval of approximately one week during February to March. Sites with high wasp numbers could not be used for translocations, as it was likely that many of the released butterflies would be killed. Wasp numbers were determined to be high when the number of wasps caught in the wasp monitoring traps showed a sharp increase, by a factor of five or more over a period of a week.

4.2.4 Vegetation surveys

Vegetation surveys were performed at each of the 15 sites between February and April 2013 in order to be able to compare the vegetation composition at sites with and without *O. ptunarra* to determine the floral requirements of the butterflies. At each site, four vegetation surveys were performed along the same transect line as that used for butterfly surveys (a total of 60 surveys altogether). The start point of each vegetation survey was selected using a random number table. Each vegetation survey was 10 m long. The vascular plant species present, the height of each plant, and the presence of bare ground, leaf litter, lichen, moss or scats were recorded at each 0.5 m point interval along the 10 m transect. Everything directly under the point was recorded. Plant species were identified to species level where possible, and otherwise to genus if the reproductive organs necessary for identification were not present. The percentage frequency of occurrence of each vascular plant species present was then determined for each transect.

4.2.5 Data analysis

Vespid wasp numbers at release sites and monitoring sites were compared to determine whether the wasp control performed at the release sites had reduced vespid wasp numbers.

The vegetation data were used to calculate the frequency of occurrence, percentage area covered, biomass, and mean and maximum heights of each species, by site. A variable was created called 'flower richness' which was the taxon richness of dicotyledonous plants for each transect. All of the flowering plants were included, as *O. ptunarra* imagoes are generalist nectar feeders that will visit any available flower (Anderson 2001).

The presence-absence data for plant species by transect were ordinated using global non-metric multidimensional scaling with the following options: Number of starting configurations = 10 (All starts generated using uniform random coordinates); Seed for random numbers = 500344; Minimum no. of dimensions = 1; Maximum no. of dimensions = 4; Maximum no. of iterations = 200; Stress ratio stopping value = 0.999900; Small stress stopping value = 0.010000; Solution scaling option = 2 (Half-changes).

The scree plot indicated a three-dimensional solution (stress = 0.19535). ANOVA was used to determine if any of the three axis scores varied between transects at sites with and without *O. ptunarra.* Pearsons product moment correlation coefficient was used to determine if the flower richness covaried linearly with any of the axis scores.

Individual plant species were analysed using ANOVA to determine whether they were predictive of *O. ptunarra* presence or absence at the sites. Many species had very small sample sizes, rendering them invalid for analysis.

4.3 Results

4.3.1 Translocation

The collection and transportation of live butterflies and eggs was performed successfully, with only one butterfly dying during transportation and three dying during the egg collection process. Mortality was due to unknown causes. The numbers of eggs collected specifically for translocation varied considerably between the years, declining from 76 eggs in 2011 to 12 eggs in 2012. These numbers do not include the eggs laid en route.

A new population of *O. ptunarra* was successfully established at Dairymaid North Plain in northwest Tasmania through translocation. New *O. ptunarra* populations were not detected at the other three release sites and these translocations were deemed to have been unsuccessful.

Monitoring surveys performed in late March 2010 at Dairymaid North Plain tentatively observed *O. ptunarra*, with one solitary female butterfly observed, but not caught for identification, which cast some doubt as to whether it was *O. ptunarra* or the closely related species *O. lathoniella.* Positive identifications of two female and six male *O. ptunarra* made in 2011 confirmed that the 2009 translocation to this site had been successful. Further *O. ptunarra* were observed at this site in low numbers in monitoring surveys performed in the 4 years following the translocation (Table 4.3). No *O. ptunarra* were observed during surveys prior to translocation or immediately after translocation at the site in 2009. Vespid wasps were in low numbers at this site during the full period of observation.

Table 4.3 Highest numbers counted along transect and densities per hectare of *O. ptunarra* butterflies and introduced vespid wasps at Dairymaid North Plain release site in 2009-2013

Year	Butterflies (Highest count)	Wasps (Highest count)	Butterflies/ha	Wasps/ha
2009	0	2	0	11
2010	1	15	9	80
2011	8	6	43	32
2012	5	4	27	21
2013	9	4	48	21

4.3.2 Wasp control

The number of wasps per hectare increased significantly over the three years at the release sites (one-way ANOVA, $F_{2, 67} = 4.93$, $P = 0.010$) (Fig. 4.2). At monitoring sites, the variation in wasp numbers was also significant, with numbers being very low in 2011, rising to their highest level in 2012 and decreasing slightly in 2013 (one-way ANOVA, $F_{2, 58} = 8.27$, $P = 0.001$) (Fig. 4.2). The wasp control at the release sites did not reduce wasp numbers to lower levels than at the monitoring sites.

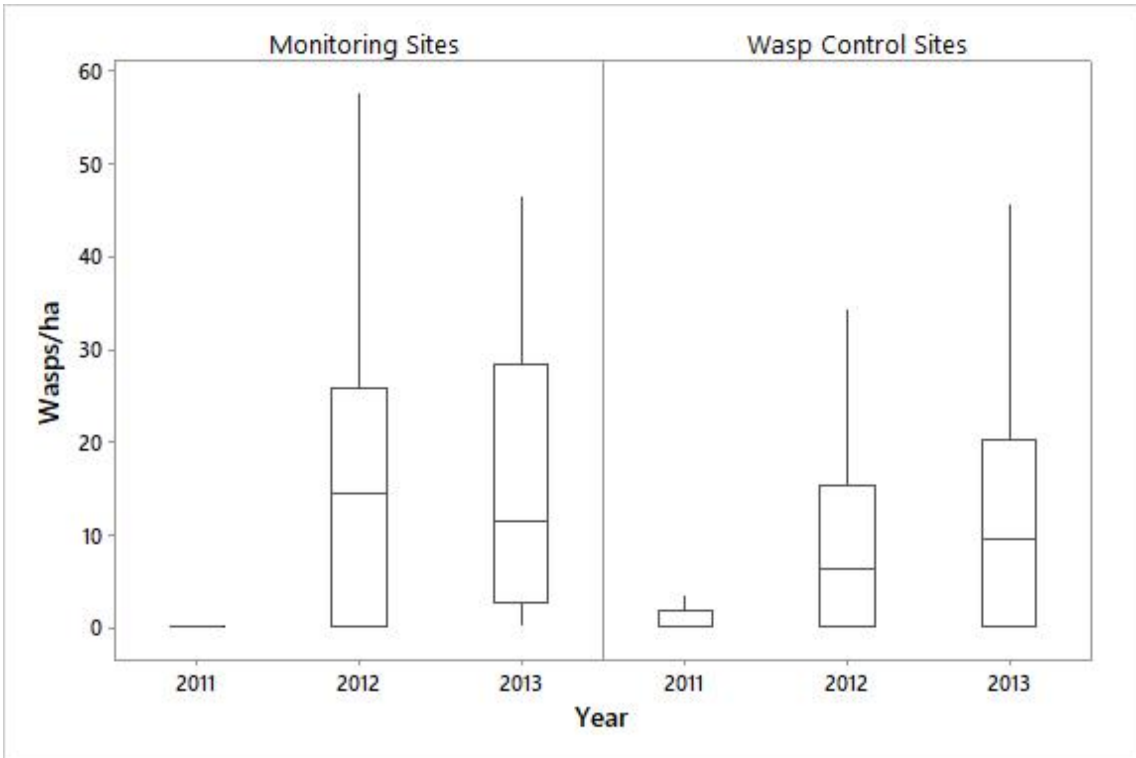


Fig. 4.2 Boxplot of the abundance of vespid wasps per hectare at the monitoring sites vs the wasp control sites in the period 2011-2013

4.3.3 Vegetation surveys

Axes 3.1 and 3.3 of the vegetation ordination had no relationship with *O. ptunarra* or with flowering plant abundance. The presence or absence of *O. ptunarra* at the sites was strongly related to axis 3.2 of the ordination (one-way ANOVA, $F_{1, 59}$ = 13.00, P = 0.001), which in turn was strongly related to flower richness (one-way ANOVA, $F_{16, 59}$ = 2.02, P = 0.034).

Sites with a greater flower richness are the sites more likely to have *O. ptunarra* present (Fig. 4.3). There were more flowering taxa present at sites where *O. ptunarra* was present, at the site where the translocation was successful and at the site where the butterflies had been observed but later became extinct (Fig. 4.3). There were fewer flowering taxa at the sites where butterflies were absent and where the translocations were unsuccessful. There was a significant negative relationship between flower richness and sites where *O. ptunarra* were absent (one-way ANOVA, $F_{4, 59}$ = 4.05, P = 0.006).

The majority of the plant species were not predictive of *O. ptunarra* presence/absence, except for some *Poa* spp. Even though *O. ptunarra* is often observed feeding on *Xerochrysum subundulatum*, the relationship was not significant (P = 0.052). Animal scats were also not significant in predicting *O. ptunarra* occurrence.

At sites where *O. ptunarra* were present there was significantly more *Poa hiemata* (one-way ANOVA, $F_{1, 59}$ = 4.48, P = 0.039) and significantly less *Poa fawcettiae* (one-way ANOVA, $F_{1, 59}$ = 21.06, $P \leq$ 0.001). *Poa labillardierei* was found to be present in similar quantities at sites where *O. ptunarra* were both present and absent.

O. ptunarra were often seen feeding from *Xerochrysum subundulatum* (orange everlasting daisy) flowers at the sites and it was theorised to be an important source of nectar for the butterflies. However, the presence of *X. subundulatum* did not have a significant effect on the presence of *O. ptunarra* (one-way ANOVA, $F_{1, 59} = 3.94$, $P = 0.052$).

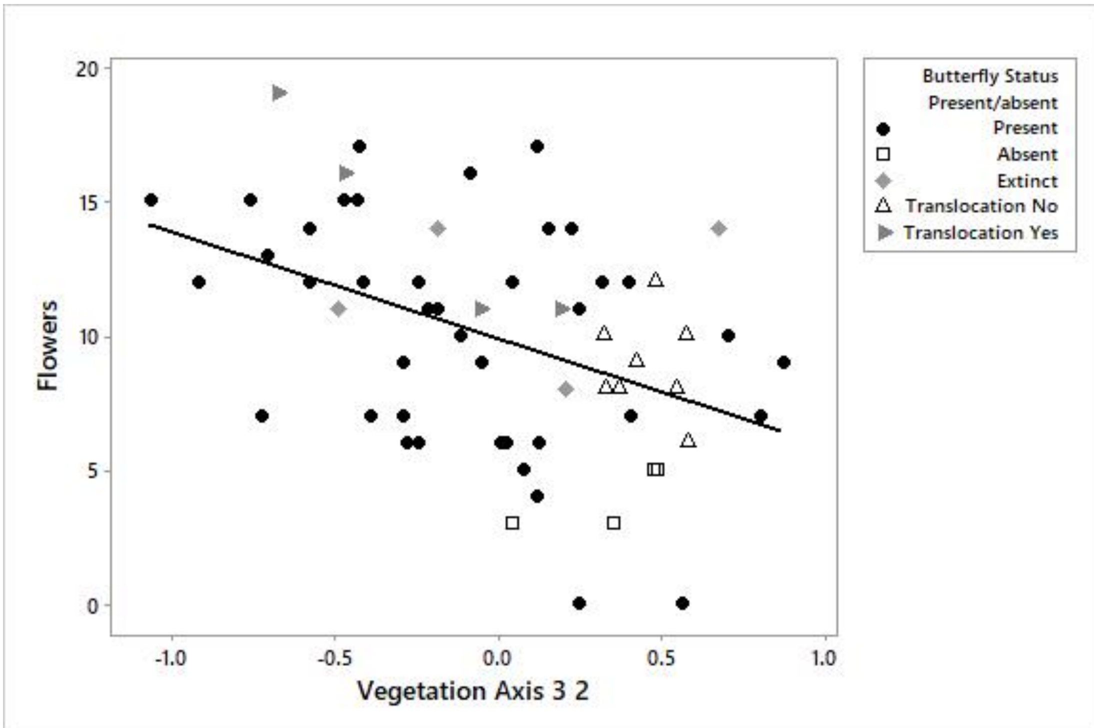


Fig. 4.3 The relationship between the flower richness and scores on axis 3.2 of the vegetation ordination, by transect, at sites where *O. ptunarra* butterflies were either present, absent, extinct, unsuccessfully translocated or successfully translocated [regression: vegetation axis 3.2 = 0.469 – 0.0473 flowers]

4.4 Discussion

A new population of *O. ptunarra* was successfully established at Dairymaid North Plain in northwest Tasmania through translocation. A successful translocation is generally classified as one where the species survives at least 3 years in the wild (Oates and Warren 1990), with *O. ptunarra* being detected in monitoring surveys performed 4 years afterwards. New *O. ptunarra* populations were not detected at the other three release sites. Those translocation attempts were deemed to have been unsuccessful.

The new population of *O. ptunarra* at Dairymaid North Plain was almost certainly the result of the translocation, as surveys before translocation did not detect the presence of this butterfly species. Other less likely possibilities include: that the population naturally re-established itself, or that a population was already present before translocation in such low numbers that it was undetectable. These are less likely scenarios, as *O. ptunarra* rarely re-establishes itself, since it is a weak flyer and the distances between isolated grasslands are large. Similarly, it is unlikely that *O. ptunarra* was already present in low numbers, as the pre-translocation surveys did not detect them, and these surveys were thorough, which is evidenced by the species being observed in the first year after translocation when numbers were very low.

Equally, there is a low possibility that *O. ptunarra* did become established at the unsuccessful release sites but were present in numbers too low to be detected. However, this is also considered unlikely, as they were not detected by the rigorous post-translocation survey effort.

The 25% success rate of the *O. ptunarra* translocations in Tasmania is comparable to the 26-38% success rate of 323 butterfly translocations performed on various species in Britain and Ireland (Oates and Warren 1990). The main reasons for successful butterfly translocations in Britain and Ireland were: the suitability of the habitat at the release site, subsequent site management and repeated translocations over several years. Some of the causes of unsuccessful butterfly translocations were: releasing only males, releasing very young larvae or eggs, release during bad weather or in a cool, wet summer, or releasing outside of their climatological range (Oates and Warren 1990). Our results will enable improved selection of sites for translocation of *O. ptunarra*. As long as management of the sites to reduce invading shrubs and predatory wasps continues, future translocations of this species should be successful.

The fact that only four out of 550 butterflies (<1%) collected in the period 2009-2012 perished during the translocations indicates that the methods used were suitable, with most butterflies and eggs being successfully collected, transported and released without mishap.

It is possible that the butterflies captured for egg collection would have oviposited larger numbers of eggs if a cutting of their host plant had been placed in the vial as well. When a cutting of *Poa* was placed with *O. ptunarra* butterflies captured for egg collection for a laboratory study, they laid an average of 4 eggs each, which is equivalent to a total of 400 eggs per 100 butterflies (Anderson 2010), which is substantially higher than the numbers of eggs collected in this study. The future collection of eggs from *O. ptunarra* butterflies would benefit from the addition of a cutting of the host plant to potentially stimulate oviposition.

The decrease in the numbers of eggs collected for translocation is probably due to collecting females for egg collection late in the season when they had already laid most of their eggs. The translocation and egg collection were performed a couple of days later in March each year from 2009-2012 due to time constraints. By 2012 the translocation and egg collection were undertaken approximately a week later (27-29 March) compared to the translocation in 2009 (20-25 March). Towards the end of March *O. ptunarra* numbers decline until the end of the flight period in early April. The peak of the egg-laying period had likely passed by the time the eggs were collected in late March 2012. It is advisable for future egg collections to take place about a week before the end of March.

The conservation management of *O. ptunarra* through translocation should be performed with caution and used only as an interim tool, while other conservation actions are being refined. Although it is relatively easy to move individuals to different locations, translocation does not address the underlying threats to the species such as predation by vespid wasps, habitat loss and fragmentation, which must be rectified to effectively conserve the species. If the underlying threats are not addressed, then it is highly likely that the newly translocated populations will also eventually succumb to these threats and become extirpated.

Although control of introduced vespid wasps was undertaken at the release sites when wasp numbers became high, it was not effective at significantly reducing their numbers in this instance. It is very difficult to fully eliminate wasps from an area, due to the effective annual dispersal and ingress of new queens. Consequently, predatory vespid wasps will remain at these sites and continue to be present in the future. To safeguard the newly established *O. ptunarra* population from vespid predation it would be advisable to continue wasp control efforts at Dairymaid North Plain. However, previous vespid wasp control in this area, using the same methods, was only 65.7 % efficient at decreasing wasp numbers and only led to a 10.1 % increase in *O. ptunarra* butterfly numbers (Potter-Craven et al. 2018). Thus, it would be advisable to increase the control effort to better protect the butterflies at this site.

Ways to increase the wasp control effort were recently described in a related study by Potter-Craven et al. (2018). The most cost-effective method is to use clustered bait stations which kill a large number of wasps in a short time with less effort required than standard intensive grid baiting systems (Harper et al. 2015). This method would be worthwhile to trial at the Surrey Hills grasslands for a greater reduction in wasp numbers with only a minimal increase in effort (Potter-Craven et al. 2018). It could also be beneficial to add an attractant such as heptyl butyrate to the baits to increase the rate of wasp visitation, which was found to significantly increase visitation by *V. pensylvanica* to fipronil baits in Hawaii (Hanna et al. 2012). Potentially, alternative toxins could be tested, such as the insect growth regulator fenoxycarb, which kills larvae and also inhibits egg production but does not directly affect the workers, enabling them to continue collecting poisoned bait and delivering it to the nest (Banks et al. 1988).

There are also some emerging techniques that could be useful for the widespread control or eradication of vespid wasps (Lester and Beggs 2019). These include: introducing the bacterium *Wolbachia* into pest insect populations that are already close to a critical Allee threshold due to previous control efforts, in order to eradicate them (Blackwood et al. 2017); and using more controversial genetic modification by inserting gene drives that induce infertility, which would inevitably lead to nest failure (Dearden et al. 2018).

It is doubtful that increasing the numbers of butterflies and eggs translocated will increase the chances of translocation success. A large review of over 300 translocations in the United Kingdom determined that translocation successes often resulted from moderate numbers of 30-50 adults or large numbers of larvae, with no correlation between the release of high numbers of adults (>50) and success (Oates and Warren 1990). The main reason for translocating larger numbers is to maintain genetic variability, with a study of the large blue butterfly (*Phengaris arion*) showing high genetic diversity 19 generations after translocating >250 larvae to sites in the UK and Sweden, to expand the butterfly's range and reduce its threatened status (Andersen et al. 2014). It is noteworthy that the movement of small numbers of *Boloria eunomia* (Esper, 1799) in France (4 and 14 butterflies to two sites) led to low genetic diversity and the loss of an allele in the ensuing populations (Barascud et al. 1999).

It is undesirable to remove many threatened butterflies from a site for translocation trials, as this may lead to the depletion of the local population. Thus, by moving both imagoes and eggs, we have attempted to keep the impact on the source site low, while maintaining the genetic diversity at the release sites.

Most successful translocations occur when suitable habitat is present at the release site and there is supporting site management (Oates and Warren 1990). Consequently, it is more likely that choosing sites with the type of vegetation composition that the butterflies prefer would lead to greater chances of translocation success and of the butterflies becoming established.

Poa tussocks need to be present, as these host the immature stages of *O. ptunarra*. Although the quantity of *Poa* spp. is similar at sites with and without *O. ptunarra*, our analyses indicate that it would be worthwhile to utilise sites dominated by *Poa labillardierei* and *Poa hiemata* for future translocations. Anderson (2010) found that *O. ptunarra* also showed a preference for *Poa hiemata* under laboratory conditions.

Although the presence of flowering nectar sources was previously not thought to be essential for *O. ptunarra,* our data indicates that nectar sources may be more important for population persistence than first thought. The positive relationship between flower richness and translocation success (Fig. 4.3) indicates that *O. ptunarra* imagoes require nectar to successfully breed and lay sufficient eggs for the population to remain viable. *O. ptunarra* is a nectar generalist that will feed from any available flowers (Anderson 2001). Previously, it had been assumed that, while a source of nectar was desirable for the butterflies, it was not necessary for their survival and that they would continue to breed even without one (Anderson 2001). However, nectar availability has also been shown to increase the longevity of *O. ptunarra* populations, with more time spent on the wing and increased individual survivorship (Anderson 2001). It is probable that nectar sources were previously present at the *O. ptunarra* sites but that the flower richness had declined due to changes in the vegetation due to sites becoming overgrown, such as shading. It is likely that a population of *O. ptunarra* may persist for a period without available nectar but that eventually their numbers will decline, leading to local extinction at that site. With the added pressures of predation by vespid wasps and increased variability in rainfall due to climate change, the presence of nectar at sites may become more important to the survival and persistence of *O. ptunarra* than previously thought.

Most Lepidoptera, including *O. ptunarra*, have functional mouthparts in the imago stage and require carbohydrates such as nectar to provide them with additional energy to reproduce and promote longevity. A recent study in Belgium of the meadow brown butterfly (*Maniola jurtina* L.), found that the females in grasslands with high quantities of nectar had higher body mass compared to those at sites with low nectar quantities. In addition, up to 40% of the females showed complete reproductive failure at sites with low quantities and low quality of nectar compared to approximately 7% of females at other sites (Lebeau et al. 2018). Nectar-poor conditions had a strong negative effect on body condition, flight activity and lifetime survival of *M. jurtina* compared to butterflies under nectar-rich conditions, with the female lifespan reduced by 22% (Lebeau et al. 2016), indicating that a greater abundance of flowering plant species present at sites would also benefit this species.

The intake of nutrients from sources other than floral resources may also be important for *O. ptunarra*'s diet. For some butterflies, drinking from mud puddles and decaying organic matter has been shown to be an important source of minerals such as sodium and nitrogen which are required for flight and egg production (Beck et al. 1999; Boggs and Jackson 1991). Such nutrients are often depleted in the soils of areas that experience high rainfalls (Ross and Dykes 1996) and are consequently low in nectar in these areas. The north-west plains of Tasmania also experience high levels of rainfall, which could potentially leach minerals from the rich basaltic soils. Mud puddling has not been observed in *O. ptunarra* and further study would be required to determine firstly if this activity occurs and then any subsequent effects on egg production.

Interestingly, at Thompsons Park Plain, the translocation site where *O. ptunarra* had recently become locally extinct, there was still a locally high flower richness. This indicates that *O. ptunarra* did not become extinct due to lack of nectar at this site. The high numbers of vespid wasps present at this site before wasp control attempts suggest that predation is likely to have been the main cause for *O. ptunarra*'s demise in this location.

4.5 Conclusion

Oreixenica ptunarra was successfully translocated to one of four release sites in the highland *Poa* grasslands of northwestern Tasmania, with a persistent population of butterflies being detected during monitoring surveys conducted in the following four years. Analysis of the vegetation composition at the sites determined that *O. ptunarra* were present at *Poa* highland grasslands sites containing the species *Poa labillardierei* and *P. hiemata* and a high abundance of flowering nectar plant species. These attributes should be taken into consideration during the selection of potential sites for future translocations of *O. ptunarra.* Translocations should be performed with caution and used only as an interim tool, while other conservation actions are being developed to effectively protect the species from the threat of predatory wasps, habitat loss and degradation, and fragmentation. Ongoing management of the sites should also be continued to reduce invading shrubs and control predatory vespid wasps.

Chapter 5 Optimal width of buffer zones around Poa grasslands to protect threatened Oreixenica ptunarra butterflies from predatory vespid wasps

Abstract

The *Poa* grassland habitat of threatened ptunarra brown butterflies *(Oreixenica ptunarra)* in north-western Tasmania, Australia, has been severely reduced through habitat loss, with the remaining fragments being interspersed with varying proportions of plantation forest and native vegetation. The fragments are vulnerable to negative edge effects, such as spillover predation from introduced vespid wasps (*Vespula germanica* and *V. vulgaris*) nesting in adjacent plantation forests. Vespid wasps have been shown to reduce numbers of *O. ptunarra* and have the potential to cause local extinctions. The optimal size of buffer zones required around *Poa* grasslands containing *O. ptunarra* populations to protect them from vespid wasps was investigated using GIS to measure the percentage of vegetation covers in buffers of varying width and relating these to average populations of wasps and butterflies. *O. ptunarra* were negatively related to plantation forest cover, while vespid wasps were positively related. Correlation analysis indicated that a native vegetation buffer around the grasslands of 300-500 m would protect the butterflies to the greatest degree.

5.1 Introduction

The loss of natural habitats due to the clearing of native vegetation for land uses such as agriculture, forestry plantations and urban expansion has resulted in species extinctions and range contractions over the last two centuries (Newbold et al. 2016; Pimm et al. 2014) and is one of the primary reasons for the decline in biodiversity worldwide (Haddad et al. 2015). These landscape changes not only reduce the total area of habitat but generally also fragment the remaining habitat into smaller remnant patches (Herse et al. 2018). As these habitat fragments are often surrounded by the converted landscapes with intensified land uses, there are frequently negative edge effects along the border between the natural fragment and the adjacent converted habitat, which further compound the negative effects of alterations in the ecosystem (Frost et al. 2015).

Negative edge effects include increased predation risk, interspecific competition, and parasitism (Herse et al. 2018; Pérez-Rodríguez et al. 2018), which can result in a further decline in biodiversity in the habitat fragment. Edge effects are frequently due to foraging or dispersing animals spilling across habitat borders (Frost et al. 2015). For example, predators spilling over from coniferous forests into adjacent calcareous grasslands in Germany resulted in higher predation rates on the grassland fragments (Schneider et al. 2013). Spillover effects between habitats are often strongest at the edges of neighbouring habitats (Schneider et al. 2013), but edge effects have been shown to extend over distances larger than 250 m, ranging up to 1 km for beetle communities in forest habitats in New Zealand (Ewers and Didham 2008).

The spillover of introduced predatory vespid wasps from areas of exotic *Pinus radiata* D. Don forest plantation is four times the magnitude of spillover from native southern beech forest in New Zealand (Frost et al. 2015). Vespid wasps are an introduced pest in many parts of the world, where they have had negative impacts on the native invertebrate fauna through predation and competition (Beggs et al. 2011). Vespid wasps are generalist predators that consume a wide variety of invertebrates and readily predate upon lepidopterans (Harris and Oliver 1993; Madden 1981). In New Zealand, lepidopterans were the second most common prey items eaten by vespid wasps in the scrubland-pastures (Harris and Oliver 1993), with predation levels high enough to reduce lepidopteran numbers (Beggs 2001) and potentially restructure lepidopteran communities in the *Nothofagus* forests (Beggs and Rees 1999).

Vespid wasps (*Vespula germanica* and *V. vulgaris*) are invasive species in Australia and have recently been observed to significantly reduce the numbers of threatened ptunarra brown butterflies (*Oreixenica ptunarra*) in Tasmania (Potter-Craven et al. 2018). *O. ptunarra* is a small orange-brown satyrine that is endemic to Tasmania. It is threatened principally due to the reduction of its *Poa* grassland habitat through land clearing, and the associated fragmentation of the remaining habitat, which the butterflies find it difficult to migrate between as they are not strong flyers. The new threat of predation by vespid wasps is of concern as a further reduction in butterfly numbers may potentially cause genetic bottlenecks and local extinctions (Potter-Craven et al. 2018). Current attempts at wasp control only led to a marginal increase in numbers of *O. ptunarra*, indicating that wasp control needs to be increased in conjunction with other conservation actions such as buffers, to effectively protect the butterflies (Potter-Craven et al. 2018).

The highly fragmented Highland *Poa labillardierei* grasslands of north-western Tasmania are habitat for *O. ptunarra* imagoes and the host plant for the juvenile stages. Some *Poa* grasslands appear to have higher numbers and healthier populations of butterflies than other sites. Sites that have a greater proportion of natural vegetation surrounding them may have fewer vespid wasps and consequently have higher butterfly numbers. It has previously been surmised that forestry operations in Tasmania, such as harvesting of plantation forests and road building, provide numerous opportunities for vespid wasps to establish nests in the disturbed ground (Bashford 2001; Bashford 2010). Similarly, high wasp nest densities of 137 nests per hectare were counted in disturbed ploughed land in New Zealand, compared to no nests in adjacent undisturbed land (Donovan 1997). Thus, *O. ptunarra* sites with large proportions of recently cleared, or newly established, plantation forest surrounding them may have higher wasp numbers. Plantation forests are highly disturbed monocultures where the previous forest has been removed, the soil has been formed into hedgerows and a new monoculture of trees has been planted which contains little understorey vegetation. Vespid wasps thrive in these disturbed conditions as there is much soft, exposed, elevated soil in which to build their nests, few predators and a convenient supply of prey within the forest and the adjacent grassland. In Tasmania, plantation forests are generally *Eucalyptus nitens* (H. Deane & Maiden) (shining gum), *E. globulus* Labill. (blue gum) or *Pinus radiata* (radiata pine).

In South Africa, plantation forests of *Eucalyptus*, *Pinus* or *Acacia* were found to be a major threat to adjacent native grassland biodiversity (Pryke and Samways 2012). It was determined that maintaining native forest within and surrounding the reserves protected both native grassland and native forest species, as well as helping to maintain biodiversity across the timber production landscape.

Edge effects, such as the spillover of predators from plantation forests, can be mitigated by placing buffer zones around the outside of nature reserves to protect the core area. The edge effects will not be eliminated altogether by buffer zones, rather they will be moved farther away from the reserve's centre to protect the biodiversity within the core area (Shafer 1999). Buffer zones can also serve to protect the core from anthropogenic threats such as hunting, mining, logging, dams, and construction by restricting those actions that are deemed to be detrimental if undertaken next to the reserve, and allowing for benign activities to occur within the buffer zone but not the core (Martino 2001; Shafer 1999).

UNESCO implemented some of the first buffers in the 1970s around their globally distributed biosphere reserves, and continue to apply them to this day (UNESCO 2016). Buffer zones are only occasionally implemented around reserves within Australia, usually in a biological conservation or regeneration context. In Queensland, a buffer strip of rainforest species was planted into pasture surrounding a tropical rainforest remnant, which was shown to have successfully regenerated, and protected the rainforest from edge effects when re-examined after 14 years (Sonter et al. 2010). In Tasmania, buffers are used by the forest industry to protect sensitive areas such as stream sides, habitats of particular threatened species and certain types of native forest from forestry operations (Forest Practices Authority 2015; Forest Practices Authority 2019).

Buffer widths are often created without scientifically investigating the requirements of the species or vegetation communities contained within the reserve. Instead, buffer zone widths are often subjectively decided by taking into account the various factors that the buffer is being created for, such as conservation, development, industry, or access by Indigenous groups, and a best guess is made of the optimal distance required. For example, a generic buffer width of 10 km was suggested for all Amazonian Nature Reserves, as this was the furthest distance that most poachers would walk from a stream or road to access a reserve for illegal purposes (Peres and Terborgh 1995) and it was arbitrarily recommended that all buffer zones around nature reserves in China should be at least 500 m (Ma 1992 in Li et al. 1999). Only a few studies have attempted an empirical approach to creating buffers (eg. Li et al. 1999), with the shortcoming of arbitrary buffers being that the buffer zones may be too small to effectively protect the values of the reserve, or too large and utilise resources and land that may alternatively be used otherwise.

The aims of the present paper are to determine if buffers are necessary around *Poa* grasslands to protect *O. ptunarra* from predatory vespid wasps and, if so, to determine a suitable buffer width. To undertake this inquiry, we will: (i) determine the percentage of the vegetation types contained in buffers of varying size surrounding each *Poa* grassland site; (ii) correlate the percentage of the vegetation types to the average numbers of *O. ptunarra* butterflies and introduced vespid wasps present at each site to determine whether the vegetation surrounding the grasslands affects their numbers; and (iii) chart the correlations at the various buffer distances to deduce the optimal buffer width.

5.2 Methods

Fifteen highland *Poa labillardierei* grassland plains in north-western Tasmania near the township of Waratah (41.446 °S 145.532 °E, 600 m asl) were examined using ArcGIS 10.4.1 to determine the percentage of each vegetation type surrounding each grassland within a series of ten buffer zones occurring at distances of 50 m to 500 m, spaced at 50 m intervals. The vegetation in the buffers was then compared to the average numbers of *O. ptunarra* and vespid wasps present at the sites to determine whether a correlation existed between them. All 15 sites were used for the wasp analysis, while only the 10 sites where *O. ptunarra* was present were used for the butterfly analysis (Fig. 5.1).

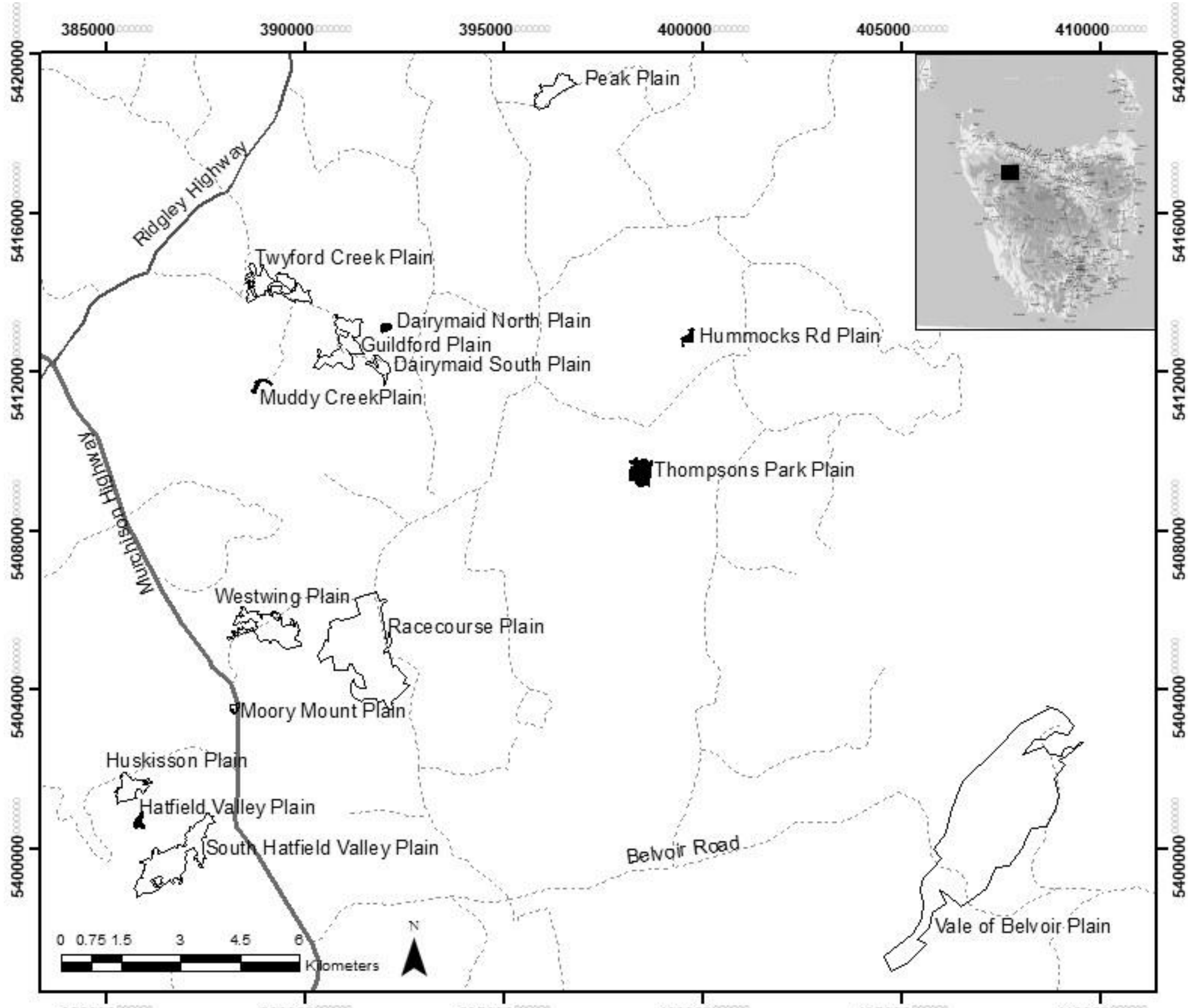


Fig. 5.1 Location of the 15 Poa grassland study sites in north-western Tasmania. All 15 sites were used for the wasp analysis (black and white sites). The 10 sites where *O. ptunarra* were present were used for the butterfly analysis (white sites)

The majority of study sites occurred within the Surrey Hills area owned by a private forestry company, with one site at the Vale of Belvoir, owned by the Tasmanian Land Conservancy. The sites were interspersed between forestry plantations, clear-felled ground, grassland and moorland, and remnants of native forest vegetation. Some of the sites were protected by formal reserves, while others were informally protected by the landowner.

The study area had a cool temperate climate and received an average of 2000 - 2400 mm rainfall per year, with no month receiving less than 100 mm (Bureau of Meteorology 2011). The underlying geology of the sites within the Surrey Hills area was Tertiary basalt, with the Vale of Belvoir occurring on a mixture of basalt and glacial deposits (Land Information Systems Tasmania 2014). Native herbivores, mainly wallabies and wombats, grazed all the sites, and cattle infrequently grazed the Vale of Belvoir.

Numbers of *O. ptunarra* butterflies and vespid wasps were counted along a fixed transect at each grassland over a period of three years during the flying season of mid-February to early April from 2011 to 2013, as described in Potter-Craven et al. (2018). The average numbers of *O. ptunarra* and vespid wasps over the three-year period were calculated for each site. The average was used rather than the median, due to the high numbers of zeros present during the surveys.

The extent of the grasslands was determined and mapped within ArcGIS 10.4.1 using an aerial photo layer from LISTmap v1.1.0.14 (Land Information Systems Tasmania 2014). Ten buffers were then created around each grassland at distances of 50 m to 500 m, spaced at 50 m intervals. The TASVEG layer v3.0 from LISTmap was then clipped to each buffer, and the quantity of each vegetation community within each buffer was calculated. TASVEG is produced by the Tasmanian Government and is the standard vegetation community layer used in Tasmania. The Tasmanian flora and vegetation community naming conventions as described in Harris and Kitchener (2005b) have been followed in the present study. Euclidean buffers were used as they are effective when analysing distances around features that are concentrated in a relatively small area within the same universal transverse Mercator (UTM) zone, as is the case in the present study.

The buffer data were exported into Excel where the vegetation communities in each buffer were grouped into the following vegetation types: dry eucalypt forest and woodland; native grassland; highland and treeless vegetation; modified land; moorland, sedgeland and rushland; non-eucalypt forest and woodland; plantation forest; rainforest and related scrub; scrub, heathland and coastal complexes; water; saltmarsh and wetland; and wet eucalypt forest and woodland (Harris and Kitchener 2005b). Plantation forest was separated from other modified land to clearly distinguish the impacts of the plantation forest. Highland and treeless vegetation was grouped with moorland, sedgeland and rushland (renamed moorland/highland vegetation), and saltmarsh and wetland was grouped with water (renamed water/wetland), as the highland and treeless vegetation and the saltmarsh and wetland vegetation types only occurred at one site, the Vale of Belvoir. The percentage of each vegetation type within each buffer was measured.

Statistical analyses were performed in Minitab 18.1. The percentage of the vegetation types in each buffer zone, around each grassland, was correlated with the average numbers of *O. ptunarra* and vespid wasps at each site to determine whether the different assemblages and quantities of vegetation types would affect the numbers of *O. ptunarra* butterflies or vespid wasps present at the grassland sites. The correlation coefficient at each buffer distance was graphed, to determine the peak buffer distance. Only significant correlations were shown in the diagrams.

5.3 Results

5.3.1 Vegetation types - overall

The dominant vegetation type surrounding the sites was plantation forest, followed by wet eucalypt forest, rainforest and related scrub, moorland/highland vegetation, and dry eucalypt forest and woodland. Native grassland, scrub, heathland and coastal complexes, modified land, water/wetland vegetation, and non-eucalypt forest and woodland were less common (Fig. 5.2).

When averaged across all the sites, the percentage of plantation forest increased with increasing buffer distance, as did the percentage of rainforest and related scrub. All other vegetation types either decreased or remained similar with increasing buffer distance (Fig. 5.2).

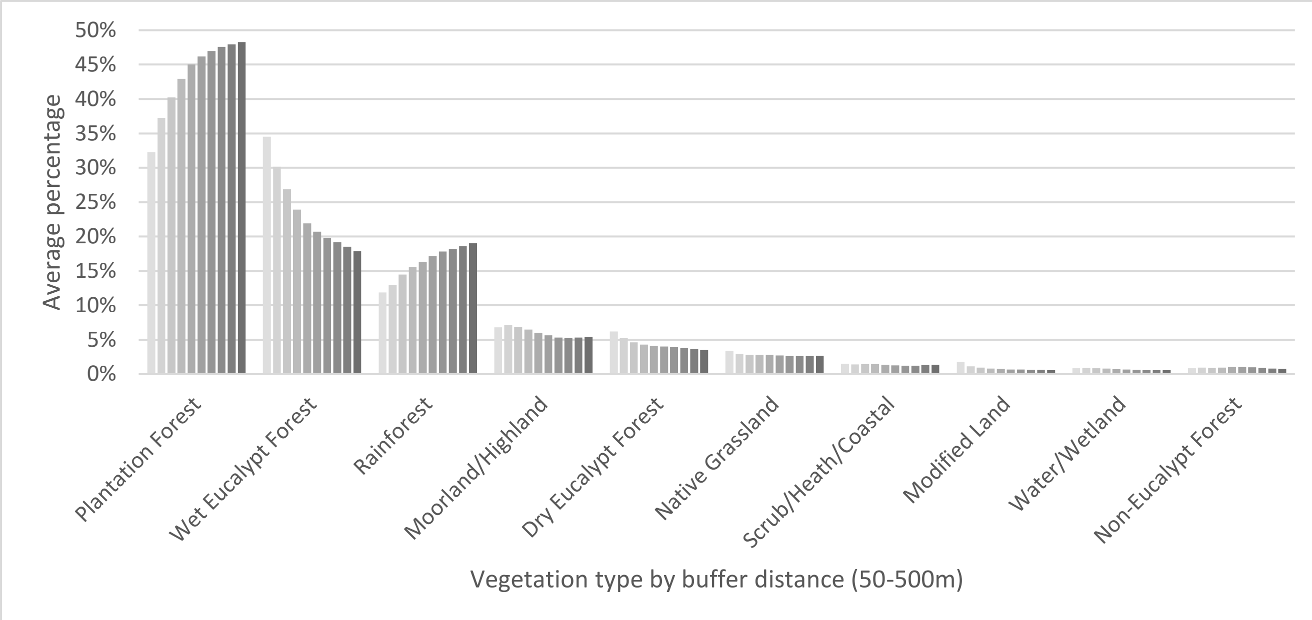


Fig 5.2 The average percentage of the vegetation types surrounding all 15 sites, at increasing buffer distances from 50-500m at 50m intervals (left to right)

5.3.2 Vegetation types – by site

The vegetation types that showed a significant correlation with *O. ptunarra* were plantation forest, dry eucalypt forest and woodland, moorland/highland vegetation, and water/wetland vegetation. Vespid wasps only had a significant correlation with plantation forest.

Most of the sites were surrounded with 0-10% dry eucalypt forest and woodland, with two sites, the Vale of Belvoir and Westwing Plain reaching a maximum of 26.2% and 37% respectively (Fig. 5.3). The amount of dry eucalypt forest and woodland increased with distance from the site for the Vale of Belvoir, with the opposite for Westwing Plain. Huskisson Plain and Peak Plain did not have any of this vegetation type within any of their buffer zones.

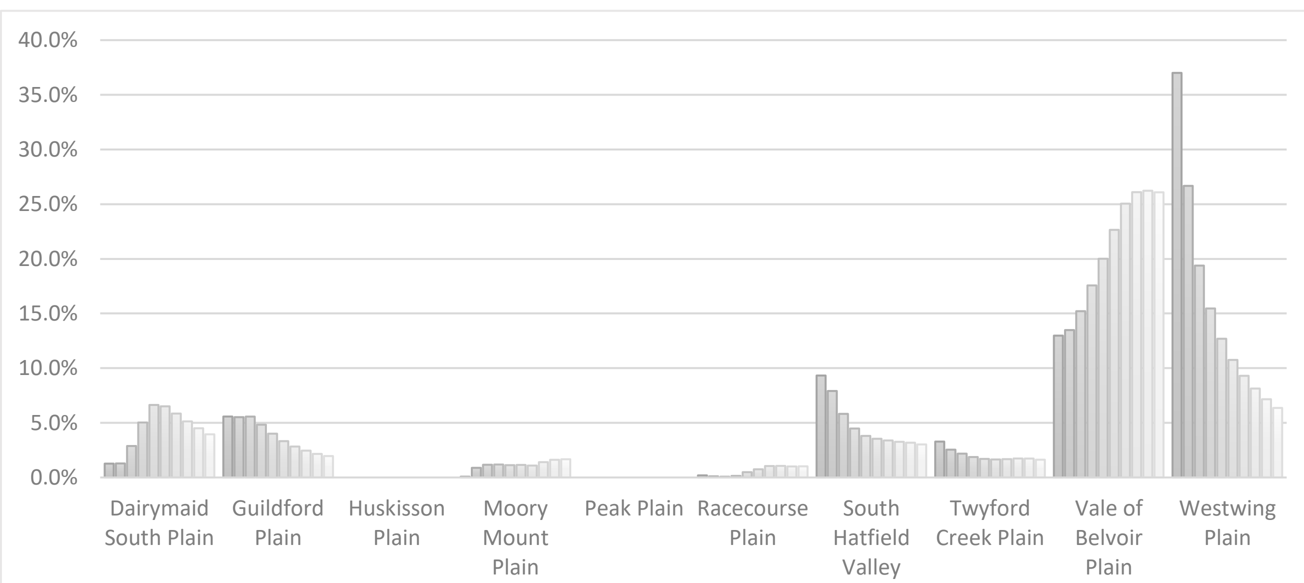


Fig. 5.3 The percentage of dry eucalypt forest and woodland vegetation within each buffer zone from 50-500 m, at 50 m intervals (left to right), by site

Most of the sites were surrounded by 0-7% moorland/highland vegetation, while three of the sites reached maxima between 22 and 43% (Fig. 5.4). Most of the sites had decreasing amounts of moorland/highland vegetation with increasing distance from the site. Peak Plain only had a marginal amount of this vegetation type within its buffer zones.

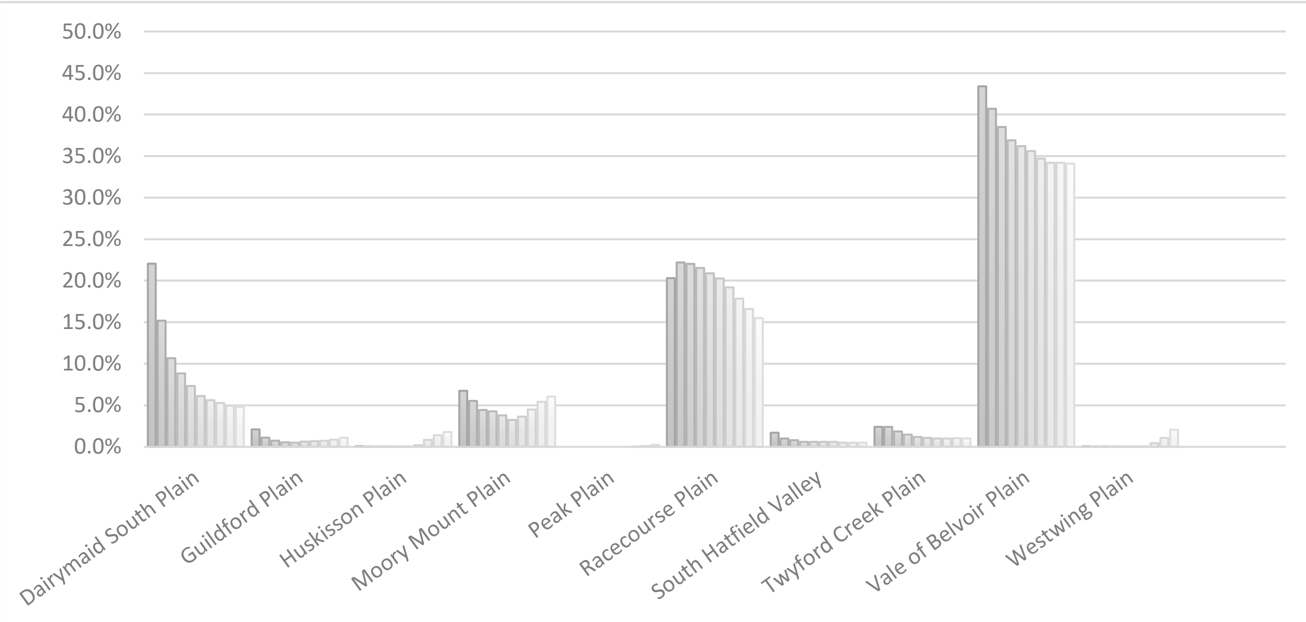


Fig. 5.4 The percentage of moorland/highland vegetation within each buffer zone from 50-500 m, at 50 m intervals, by site

Water/wetland vegetation only occurred at two sites, Twyford Creek Plain and the Vale of Belvoir, with both sites showing a trend of decreasing water/wetland vegetation with distance from the site (Fig. 5). Twyford Creek had a maximum of 3.2% of this vegetation type surrounding the site, with the Vale of Belvoir having a maximum of 11%. None of the other sites had this vegetation type within their buffer zones. As this vegetation type had a low level of replication, as it only occurred at two of the sites, its significance on *O. ptunarra* populations should be disregarded in this instance.

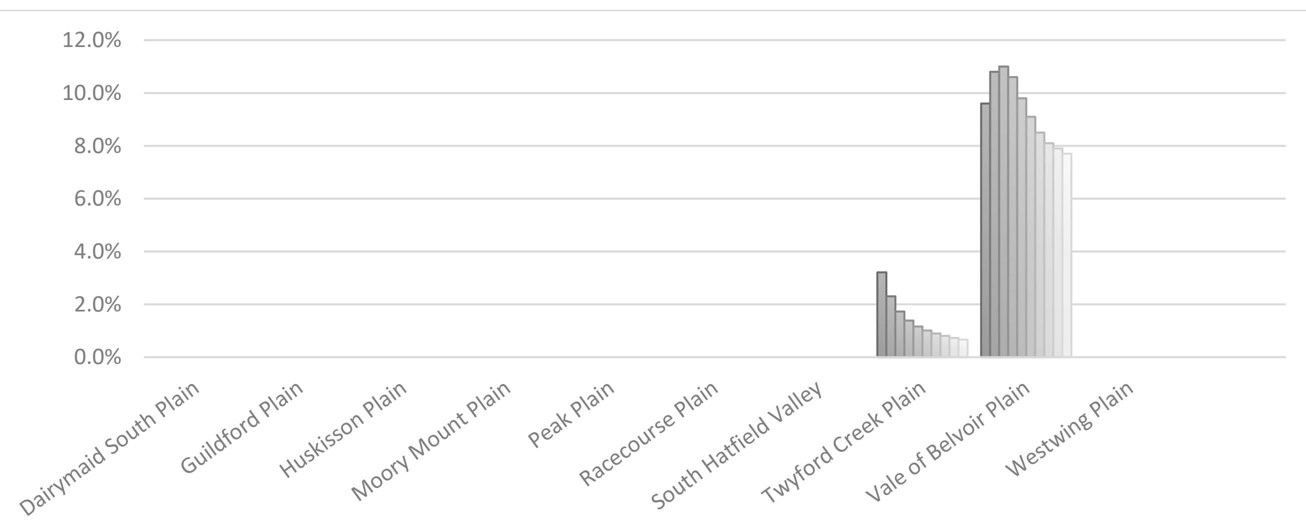


Fig. 5.5 The percentage of water/wetland vegetation within each buffer zone from 50-500 m, at 50 m intervals (left to right), by site

Most of the sites used for the butterfly correlation analysis showed an increase in plantation forest with increasing distance from the site, with maxima ranging from 15.1% to 72.9% at South Hatfield Plain and Guildford Plain respectively (Fig. 5.6). Similarly, most of the sites used for the wasp correlation analysis showed a trend of increasing percentages of plantation forest with increasing distance from the site, with maxima ranging from 15.1% to 92.5% at South Hatfield Plain and Muddy Creek Plain respectively (Fig. 5.7). The Vale of Belvoir was the only site that did not contain plantation forest within any of its buffer zones.

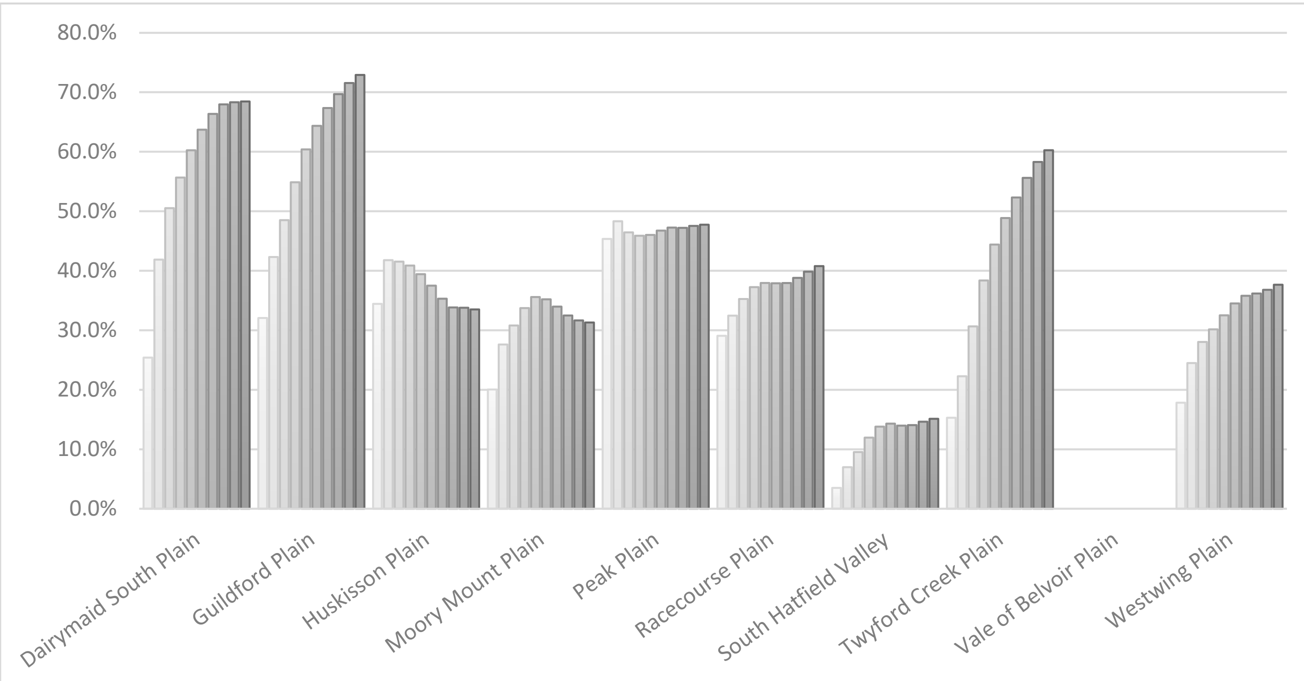


Fig. 5.6 The percentage of plantation forest vegetation within each buffer zone from 50-500 m, at 50 m intervals (left to right), at each site used for the butterfly correlations

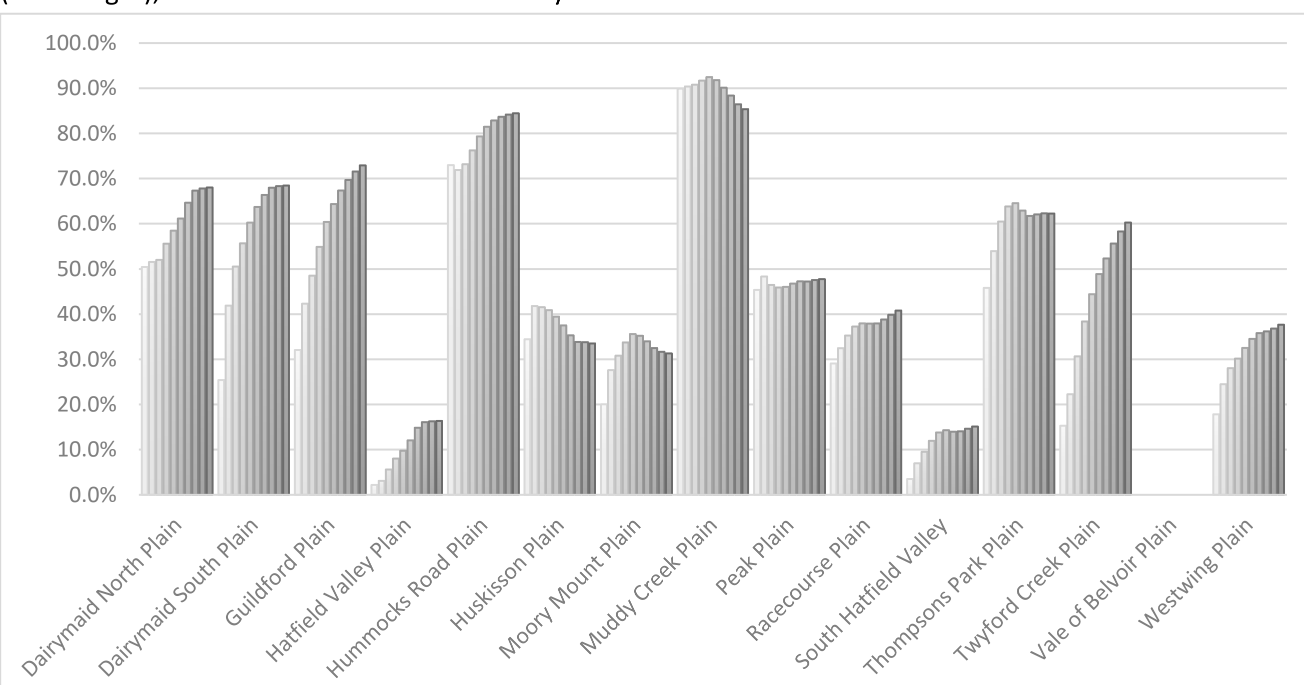


Fig. 5.7 The percentage of plantation forest vegetation within each buffer zone from 50-500 m, at 50 m intervals (left to right), at each site used for the wasp correlations

5.3.3 Correlations

There was a significant positive correlation between average numbers of *O. ptunarra* and dry eucalypt forest and woodland, and moorland/highland vegetation types occurring in the buffer zones surrounding the grassland sites. When the correlation coefficients for the various buffer zones from 50 to 500 m were graphed, there was a steady increase in the correlation between average numbers of *O. ptunarra* and the percentage of dry eucalypt forest and woodland with increasing distance from the site (Fig. 5.8). The correlation was not significant before 200 m. The correlation between average numbers of *O. ptunarra* and the percentage of moorland/highland vegetation increased steadily with increasing distance from the grassland site (Fig. 5.9).

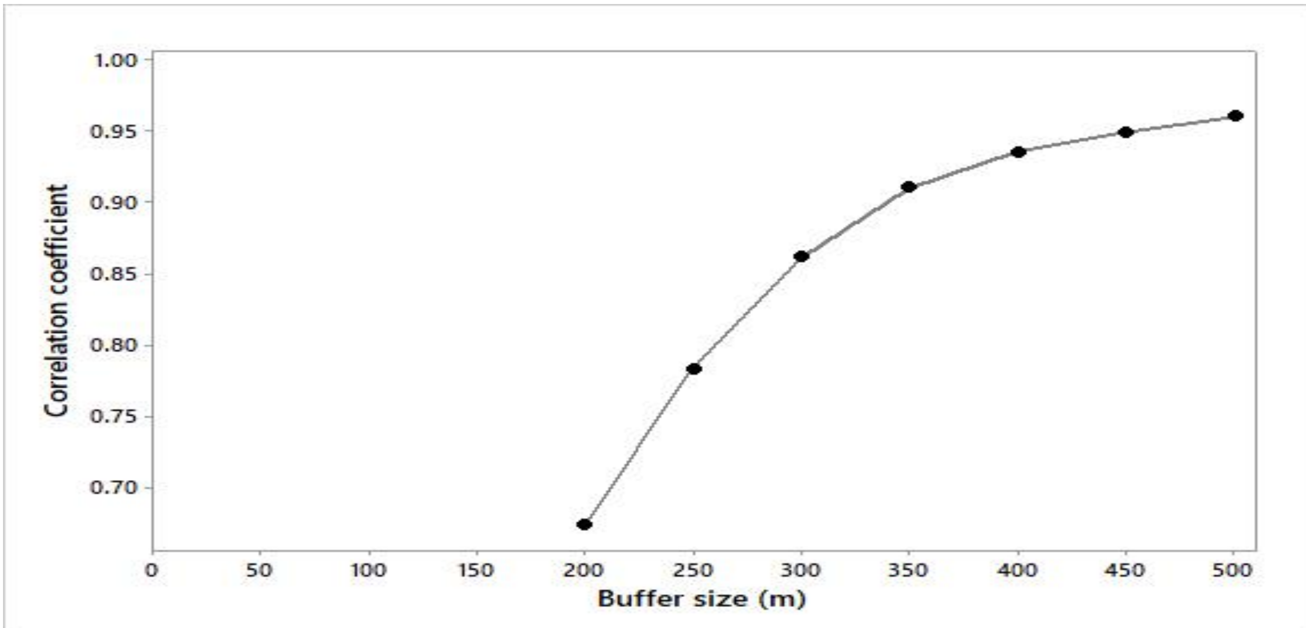


Fig. 5.8 The correlation between average numbers of *O. ptunarra* and the percentage of dry eucalypt forest and woodland at different buffer distances from 50-500 m

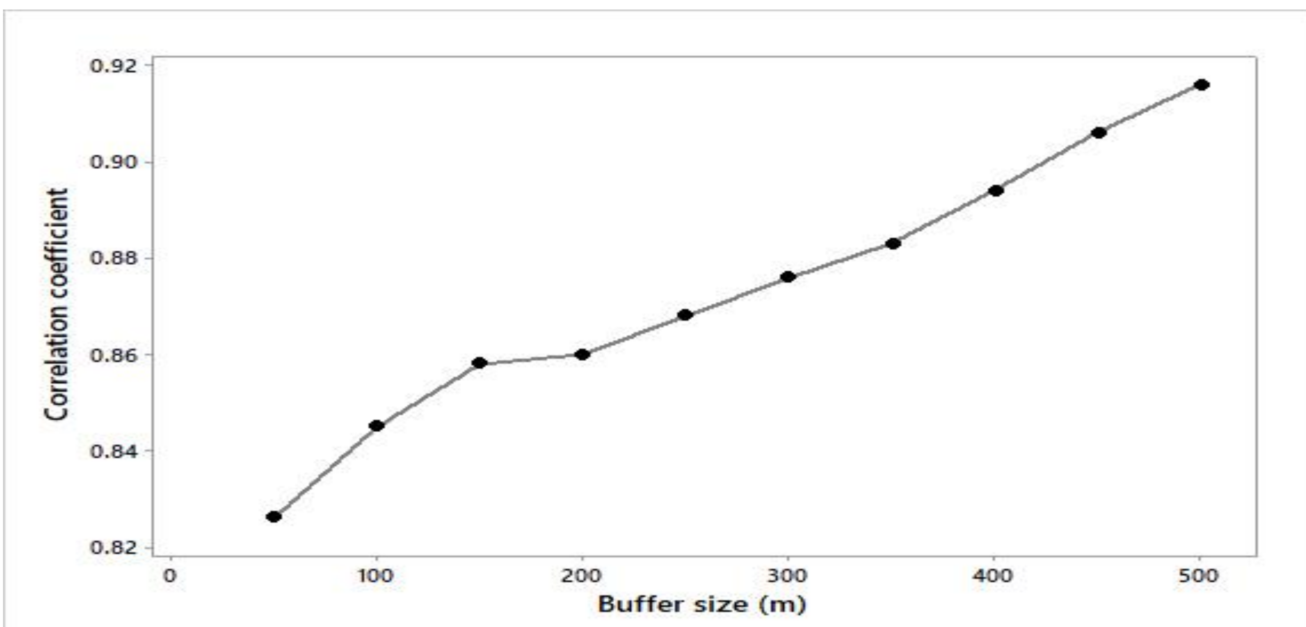


Fig. 5.9 The correlation between average numbers of *O. ptunarra* and the percentage of moorland/highland vegetation at different buffer distances from 50-500 m

There was a significant negative correlation between the average numbers of *O. ptunarra* and the percentage of plantation forest within the buffers. The correlation coefficient was highest at 250 m where it peaked at -0.625, after which it steadily decreased with increasing distance from the grassland site (Fig. 5.10). The correlation was not significant before 200 m.

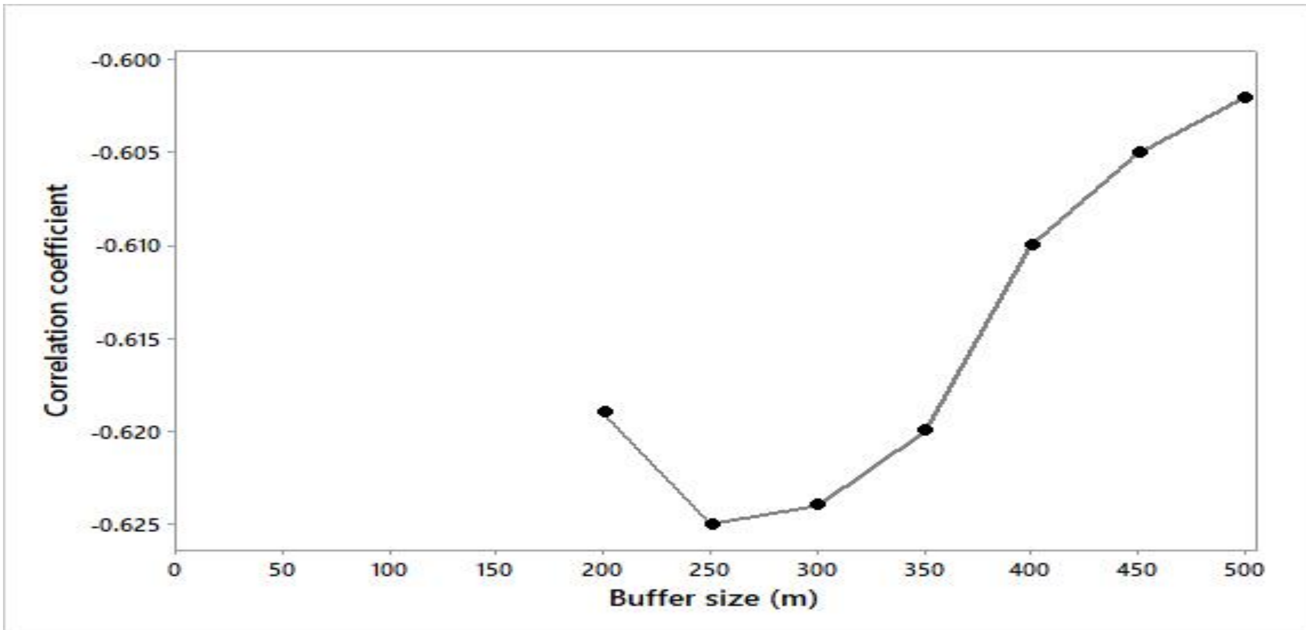


Fig. 5.10 The correlation between average numbers of *O. ptunarra* and the percentage of plantation forest at different buffer distances from 50-500 m

There was a significant positive correlation between average numbers of vespid wasps and the percentage of plantation forest within the buffers. The correlation coefficient gradually increased until it peaked between 250 and 300 m at 0.659, after which it steadily decreased with increasing distance from the grassland site (Fig. 5.11).

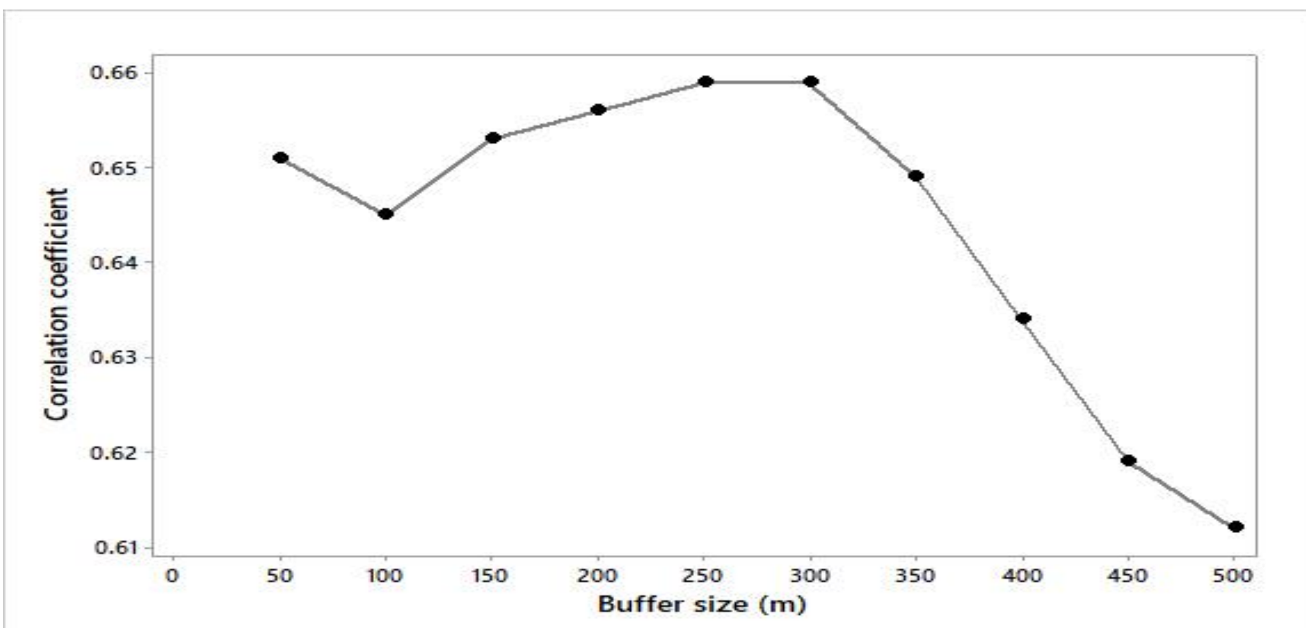


Fig. 5.11 The correlation between average numbers of vespid wasps and the percentage of plantation forest at different buffer distances from 50-500 m

5.3.4 Example site – Peak Plain

The buffers around Peak Plain, shown in Fig. 5.12 are dominated by plantation forest, which is >40 % in all of the buffer zones. The is also a large amount of rainforest present in all of the buffers (>30%). Wet Eucalypt forest is present to a lesser degree, between 9.6% and 17.6%. Modified land (road), other grassland, and scrub/heath/coastal vegetation are all present at <5% (Fig. 5.13).

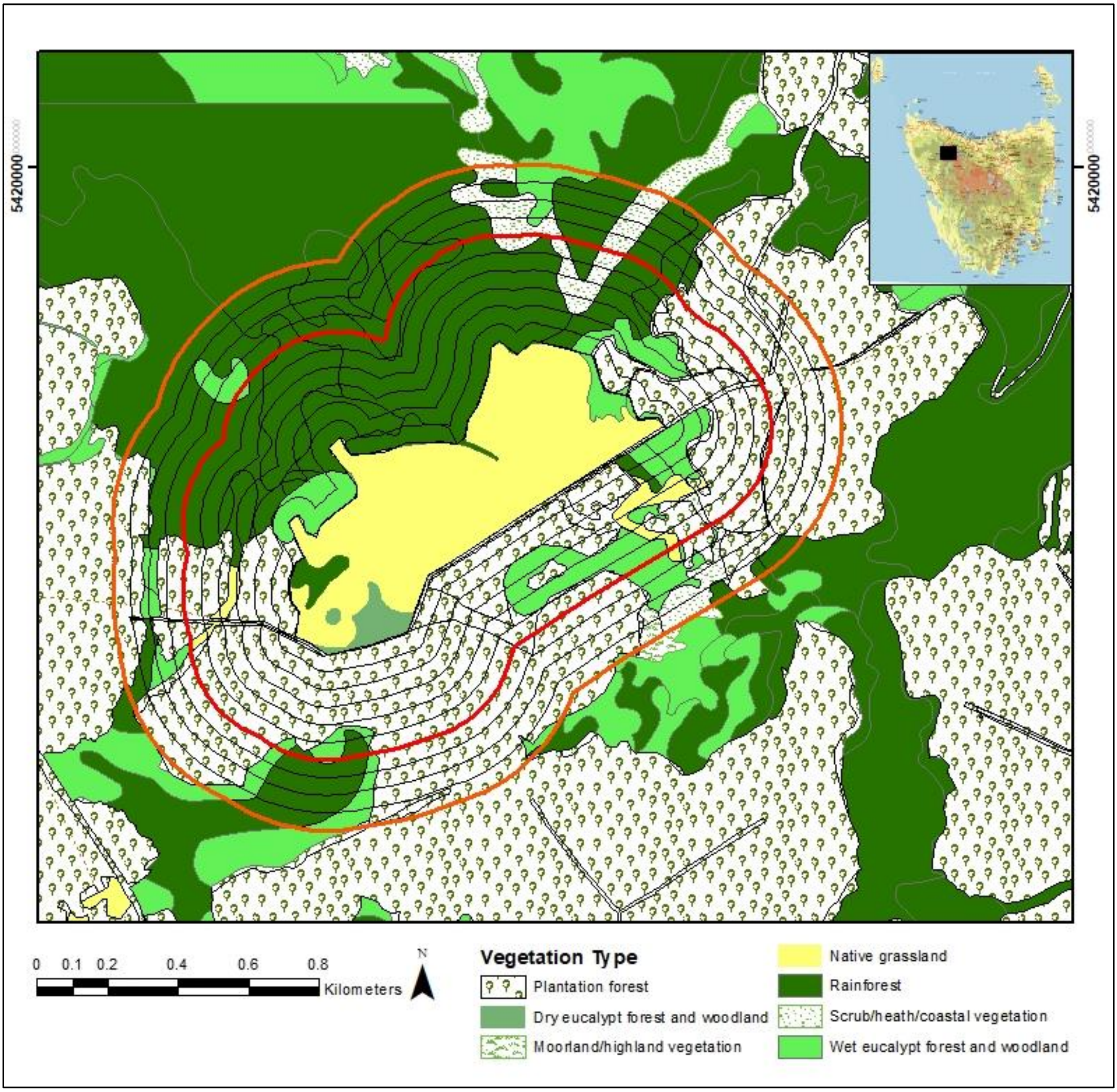


Fig. 5.12 Peak Plain grassland site (large yellow area in centre of map) showing the different vegetation types present different at increasing buffer distances from 50-500 m. The 300 m buffer (red) and the 500 m buffer (orange) have been highlighted

Native vegetation buffers to protect *O. ptunarra* from vespid wasps, could be put in place over time, for ease of implementation. Firstly, the existing native vegetation within the nominated wasp buffer distances of 300 m or 500 m should be rehabilitated if necessary and then left intact. Next, young plantation forests could be cleared and rehabilitated back to native vegetation. Lastly, when older established plantation forests are due to be harvested, the land within the designated wasp buffer zone should be cleared and rehabilitated back to native vegetation. Care should be taken to cover ground that is disturbed by harvest operations or rehabilitation works with weed matting or similar, to prevent wasps from digging nests in these areas. Works should also be performed in winter when the wasps are hibernating

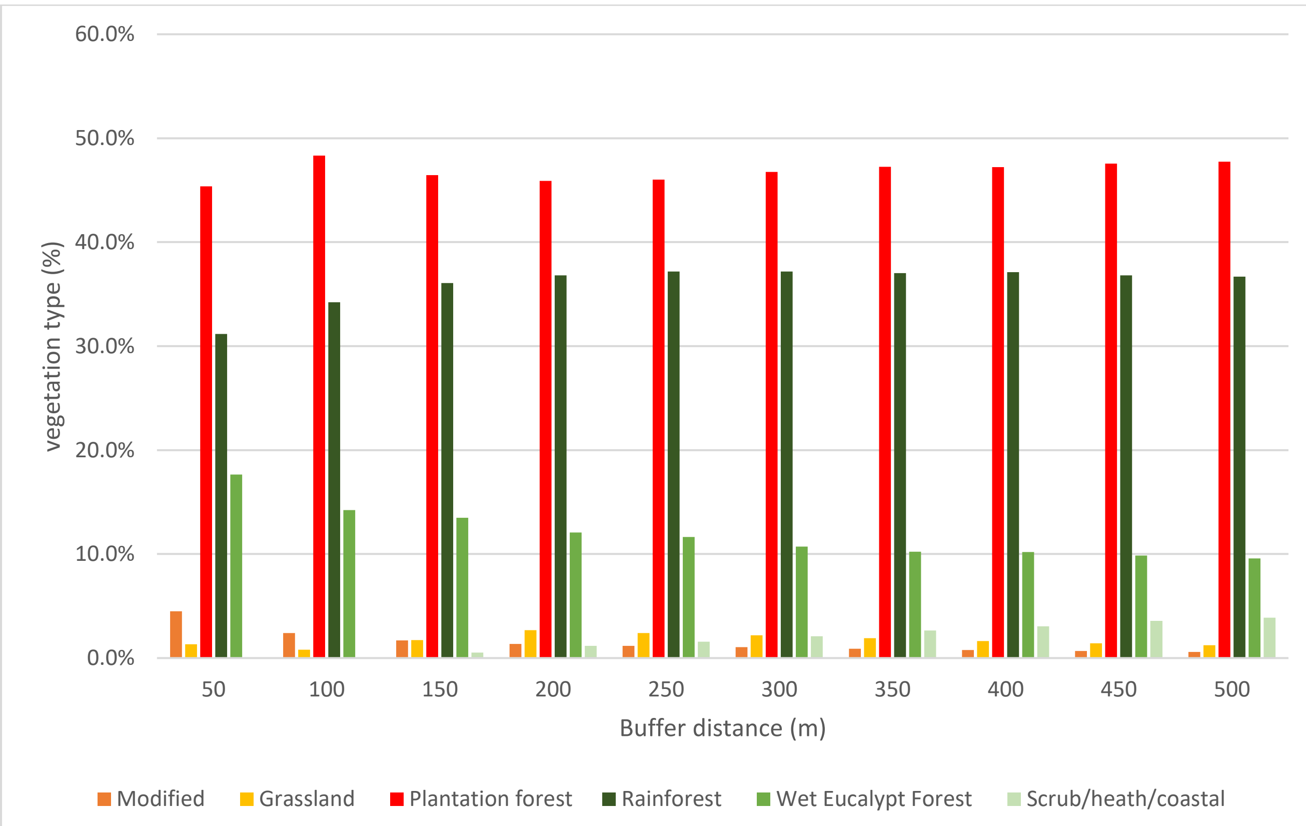


Fig. 5.13 The percentage of each vegetation type present within each buffer zone from 50-500 m, at Peak Plain

5.4 Discussion

Habitat loss and the associated fragmentation of the remaining *Poa* grasslands in north-western Tasmania has resulted in the grasslands now being surrounded by a mosaic of vegetation communities dominated by plantation forest. The negative relationship between *O. ptunarra* and plantation forest and the converse positive relationship for vespid wasps is attributed to the high densities of wasp nests within the plantation forest, and the associated spillover of wasps into the adjacent *Poa* grasslands. High numbers of vespid wasps in forestry areas in Tasmania have previously also been associated with high densities of wasp nests within the plantation forests (both pine and eucalypt), especially ones cleared recently where many nests were located in the disturbed ground (Bashford 2001; Bashford 2010). Although the numbers of vespid wasp nests within plantation forests are thought to be high, the densities remain unquantified and further studies are required. A basic population genetic analysis could be a relatively easy way of quantifying nest density in future studies.

The positive correlation between average *O. ptunarra* butterfly abundance and dry eucalypt forest and woodland vegetation is not significant until around 200 m away from the grassland sites, whereas the significant positive correlation with moorland/highland begins at 50 m. This could be related to edge effects and the differences in structural complexity between the two vegetation types. There are possibly more avian predators of butterflies present along the edge between the grassland sites and the dry eucalypt forest and woodland, than along the edge with the moorland/highland vegetation. Investigating *O. ptunarra*'s natural predators use of forest edges was beyond the scope of this study.

The correlations between the percentage of plantation forest present at various buffer distances from the grassland sites and the average numbers of *O. ptunarra* and vespid wasps present peaked at 250 m and 250-300 m respectively, indicating that the minimum buffer size around the grasslands to exclude vespid wasps should be at least 300 m. The relationship between plantation forest and both *O. ptunarra* and wasps was significant even as the correlation declined, indicating that plantation forest was still having a meaningful effect on these species. Vespid wasps generally do not fly further than 400 m from the nest while foraging (Archer 2012), so a buffer width of 500 m would better protect the butterflies than 300 m.

It is important that the buffers contain undisturbed vegetation communities, which are more difficult to build nests within and so not preferred by vespid wasps. Undisturbed pasture studied in New Zealand contained no vespid wasps nests, while adjacent ploughed land had >100 nests per hectare, with the number of suitable nest sites found to be the critical factor determining the number of wasp nests, if other factors such as weather and food availability were suitable (Donovan 1997). The logical choice of vegetation types for the buffers would be those that previously surrounded the *Poa* grasslands before the plantation forests were established, remnants of which still occur and were evident in the GIS analysis. Some of the vegetation types were positively correlated with *O. ptunarra* numbers and not correlated to vespid wasp numbers. However, the percentage cover of these vegetation types may simply reflect the degree of their loss to plantation forests rather than a strong association with *O. ptunarra*.

Ideally, *O. ptunarra* populations in *Poa* grasslands should be surrounded by a buffer of intact and undisturbed native vegetation. However, if the buffer zones already contain plantation forest or disturbed ground, then restoration will be necessary to remove the plantation forest and rehabilitate the buffer. Rehabilitation should be performed in such a way as to reduce the area of disturbed ground, discourage wasp nest building, and encourage local vegetation communities to establish.

It is possible that older plantation forests, where an understorey has developed, and the soil has compacted over time, would contain less disturbed ground for wasps to build nests. Further research is required to determine their suitability. If not suitable for wasp nests these forests could be left in place until their prescribed harvest time and then rehabilitated within the buffer zones.

Currently, controlling wasps through toxic baiting and extermination of nests to protect *O. ptunarra* from vespid wasp predation has only a small effect, and is also very time consuming, labour intensive and expensive (Potter-Craven et al. 2018). This indicates that other conservation measures are required to protect this threatened species. Buffer zones would be a positive conservation action to assist in the protection and recovery of *O. ptunarra* by excluding vespid wasps without the need for costly and laborious toxic baiting. This could be a better long-term conservation action, as currently workers are required to go out several times a year during the wasp season to place the baits. If the buffers sufficiently restricted wasp incursions, then little or no further wasp control work would be required for many years. The reduced use of poisons would also benefit the ecosystem.

Creating buffers to protect threatened *O. ptunarra* butterfly populations, many of which occur within reserves, would be consistent with international protocols, such as UNESCO biosphere reserves which are surrounded by buffers to protect important habitats within the reserves (UNESCO 2016). As *O. ptunarra* is a threatened species, populations of the butterfly outside of reserves also warrant protection. It would be advisable for the state government regulator for threatened species in Tasmania, the Department of Primary Industries, Parks, Water and Environment, and the federal regulator, the Department of Environment and Energy, to collaborate to update the long out-of-date recovery plan for *O. ptunarra* (see Bell 1999) to include some contemporary conservation actions, including buffers, to protect the butterflies from vespid wasps.

The Forestry Practices Authority (FPA), the governing body for the forest industry in Tasmania, already has policies on buffer zones around the habitat of some other threatened species. For example, active nests of Tasmanian wedge-tailed eagles (*Aquila audax fleayi* Condon & Amadon, 1954) and white-bellied sea-eagles (*Haliaeetus leucogaster* Gmelin, 1788) must have a minimum 10 ha circular buffer placed around them to protect them from disturbance during the breeding season (Forest Practices Authority 2014). Similarly, many logging coupes (commercial stands of timber) contain Wildlife Habitat Clumps (WHC), which are areas of habitat retained to protect targeted threatened flora and fauna species, as well as the biodiversity of non-threatened species (Forest Practices Authority 2010a). Features included within WHC to protect threatened species can include: trees for hollow-nesting birds, decaying logs for velvet worms (*Tasmanipatus* spp. Ruhberg, Mesibov, Briscoe & Tait, 1991), deep leaf litter patches for stag beetles (*Hoplogonus* spp. Parry, 1875) and stands of blue gum trees for swift parrots (*Lathamus discolor* (White, J., 1790)) (Forest Practices Authority 2010a). Wildlife habitat strips of retained native vegetation about 100 m wide are also retained on occasion, to protect habitat for native flora and fauna (Forest Practices Authority 2010b). Additionally, the Warra Long Term Ecological Research Site in southwest Tasmania, which is partly managed by the FPA, has previously had a 2 km wasp-free buffer along the boundary between the site and the Tasmanian Wilderness World Heritage Area to protect the reserve from incursion by vespid wasps while they were still expanding their range (Bashford 2001).

Accordingly, it is recommended that government regulators establish a policy to protect *O. ptunarra* by placing buffer zones around the *Poa* grasslands where they occur near plantation forests, to protect *O. ptunarra* from the spillover of predatory vespid wasps nesting within the disturbed ground in the plantation forest.

5.5 Conclusion

The GIS analysis of the vegetation surrounding the *Poa* grasslands of north-western Tasmania, which is habitat for threatened *O. ptunarra* butterflies, determined that they were predominantly surrounded by plantation forest. The negative relationship of *O. ptunarra* with plantation forest cover, and the conversely positive relationship of vespid wasps with plantation forest, was attributed to the high densities of wasp nests within the plantation forest, and the associated spillover of wasps into the adjacent *Poa* grasslands. The correlation analysis indicated that the optimal buffer width to place around *Poa* grassland sites to exclude vespid wasps and protect populations of *O. ptunarra* was 300-500 m. Buffer zones would be a positive conservation action for *O. ptunarra* which is under threat from predation by vespid wasps, especially as other forms of wasp control are currently only partially effective at protecting *O. ptunarra*. As buffer zones are already used to protect some threatened species in Tasmania and have previously been used to protect reserves from incursions by wasps, it is recommended that government regulators create a policy for the implementation of buffer zones around populations of *O. ptunarra*.

Chapter 6 Discussion

6.1 The impacts of vespid wasps on *O. ptunarra*

The present study is one of the first in Australia to document the impact of invasive predatory vespid wasps on a native species. It is particularly relevant as *O. ptunarra* is a threatened species whose numbers have already declined due to other threats. The additional threat of predation by vespid wasps may accelerate the decline of the species. The present study confirmed that predation by vespid wasps is significantly reducing *O. ptunarra* numbers, with higher numbers of *O. ptunarra* being detected at sites where wasp control was performed, as opposed to sites without wasp control (Chapter 3).

There has been a significant decline in population sizes of *O. ptunarra* since vespid wasps became established in north-western Tasmania in the late 1990s (Chapter 3). This association of decline with vespid wasps is similar to observed declines in populations of local invertebrates elsewhere in the world (Beggs et al. 2011). For example, in New Zealand, wasp predation was so high that caterpillars (*Uresiphita polygonalis maorialis* Felder & Rogenhofer, 1875) and orb-web spiders (*Eriophora pustulosa* Walckenaer, 1842) present during the peak of the wasp season had very little probability of surviving to the end of the season (Beggs 2001). Significant declines in lepidopteran numbers due to vespid wasp predation were also observed in New Zealand, to the extent that some lepidopteran communities were restructured (Beggs and Rees 1999).

The present study further determined that vespid wasp predation is keeping the butterflies at low densities (Chapter 3). As vespid wasps are generalist predators that switch to other prey species when the abundance of a prey becomes low, some prey species persist at low densities without becoming extinct (Beggs 2001). The abundance of prey species should rise again if wasps are removed from the community or if their numbers are sufficiently reduced (Sackmann et al. 2008).

The markedly high wasp numbers at some *Poa* grasslands have been attributed in the present study to the large availability of prime nesting sites in the disturbed ground of nearby plantation forests (Chapter 5). Wasps build high densities of nests within the soft soil within the plantation forests, resulting in a boom in wasp numbers. They then spill over from the plantation forest into the adjacent *Poa* grasslands where they predate on the native invertebrates, including *O. ptunarra.* This is similar to the spillover of predators from coniferous forests into adjacent calcareous grasslands in Germany, which resulted in higher predation rates on the grassland fragments (Schneider et al. 2013). Correspondingly, the spillover of vespid wasps out of pine plantation forests relative to native forests occurred at a high ratio of 4:1 in New Zealand (Frost et al. 2015).

The high numbers of vespid wasps present in the *Poa* grasslands and the subsequent high levels of predation on *O. ptunarra* have the potential to cause genetic bottlenecks and local extinctions of the butterflies (Potter-Craven et al. 2018). This has previously been evidenced in areas of high wasp densities in New Zealand, where the high levels of predation on native *Prolasius* ants eroded their genetic diversity, leading to a genetic bottleneck (Burne et al. 2017). The fragmentation of *O. ptunarra* habitat means that the butterflies are unlikely to recolonise sites where they have become locally extinct, as *O. ptunarra* is not a strong flyer and not able to cross the long distances between sites, often across unsuitable habitat such as plantation forests. Similarly, habitat loss, degradation and fragmentation resulted in the inability of the Quino checkerspot (*Euphydryas editha quino* Behr et al., 1863) to recolonise isolated habitat patches after populations became extinct due to climate variability in the USA (Preston et al. 2012).

O. ptunarra is a flagship species for the plight of *Poa* grassland ecology in Tasmania. It is doubtful that vespid wasps are targeting butterflies in isolation, and it is likely that other invertebrate species would also be suffering population declines due to wasp predation. Further studies are required into the impact of vespid wasps on other invertebrates and ecosystems in Australia.

6.2 Management of vespid wasps for the conservation of *O. ptunarra*

Wasp control trials using toxic baits laced with fipronil were successful at reducing vespid wasps numbers by 65.7%, which subsequently allowed for an increase in *O. ptunarra* numbers of 10.1% (Chapter 3) (Potter-Craven et al. 2018). This increase indicated that wasps keep *O. ptunarra* densities at low levels through predation and that their numbers could expand again if vespid wasps were better controlled.

Wasps in carbohydrate-rich *Nothofagus* forests in New Zealand have been successfully controlled by poisoning wasps using sodium monofluoroacetate and sulfluramid contained in cat food placed within bait stations laid out in a grid that covered the entire 30 ha site (Beggs et al. 1998). It was deduced that vespid wasp numbers needed to be reduced by 80–95% of their original numbers, which equates to between 2.7 and 5.5 wasps per malaise trap per day, in order to adequately protect native invertebrates (Beggs and Rees 1999; Toft and Rees 1998).

The wasp control effort in Tasmania would need to be increased to more effectively reduce wasp numbers, which would consequently decrease the resulting predation pressure on *O. ptunarra* and allow butterfly numbers to flourish again. In New Zealand, more intensive wasp control efforts have enabled a greater reduction in wasp numbers, which has resulted in more effective protection of the native fauna (Harper et al. 2013).

Ways of increasing the wasp control effort were outlined in Chapter 3 and include placing a higher density of traps either in a grid pattern, or by clustering bait stations together at intervals within the grasslands. Baiting along a straight line is generally not as successful as these more intensive methods, as fewer wasps encounter the traps (Harris and Etheridge 2001). Baiting in a systematic grid is likely to increase the chances of foragers from most nests encountering the bait (Harris and Etheridge 2001) but would entail an increase in labour intensity and thus more costs, as many more traps would be involved (Beggs et al. 1998). Clustered bait stations have been found to kill large numbers of wasps in a short time with less effort required than intensive grid baiting systems. An 80% reduction in wasp traffic at nests within 113 m of a cluster and a 50% reduction at nests within 250 m was recorded in New Zealand forests (Harper et al. 2015). This may be an effective technique to trial within *O. ptunarra* grasslands for a larger reduction in wasp numbers with a minimal increase in effort (Potter-Craven et al. 2018).

To increase the rate of wasp visitation to the bait stations, the addition of an attractant to the baits could prove beneficial. The addition of heptyl butyrate was found to be an effective attractant for *V. germanica* (Buteler et al. 2018) and significantly increased visitation by *V. pensylvanica* to fipronil baits in Hawaii (Hanna et al. 2012).

Fipronil is currently the most effective and commonly used toxin for vespid wasp control, with 1080 and sulfluramid also being used on occasion (Beggs et al. 1998; Harris and Etheridge 2001). The insect growth regulator fenoxycarb is an alternate toxin that has been used for the control of red imported fire ants (*Solenopsis invicta*) in the USA and could also be effective for the control of vespid wasps (Banks et al. 1988). Fenoxycarb kills the larvae and also inhibits egg production by the queen but does not directly affect the workers, enabling them to continue collecting poisoned bait and delivering it to the nest (Banks et al. 1988). By not affecting the workers, fenoxycarb could potentially be more effective than fipronil, as fipronil also causes the death of the workers.

Some emerging techniques that could be useful for the widespread control or possible eradication of vespid wasps were outlined in Chapter 4 and include: introducing the bacterium *Wolbachia* into pest insect populations that have previously been subject to control efforts and are already experiencing Allee effects, in order to eradicate them (Blackwood et al. 2017); or by inducing infertility through the use of the more controversial genetic modification technique of inserting gene drives, which would inevitably lead to nest failure (Dearden et al. 2018; Lester and Beggs 2019).

Permanent eradication of introduced vespid wasps from Tasmania is not feasible, and localised control by toxic baiting is expensive, time consuming and labour intensive. The present study determined that a more efficient and long-term solution would be to place vegetation buffers around *Poa* grasslands to reduce vespid wasp incursions into *O. ptunarra* habitat, thereby reducing the predation pressure on *O. ptunarra* (Chapter 5).

The GIS analysis of the proportion of vegetation surrounding the *Poa* grasslands showed that they are predominantly surrounded by plantation forests (Chapter 5). When the proportion of vegetation present was correlated to average numbers of *O. ptunarra* and vespid wasps, it was determined that plantation forests were having a negative effect on *O. ptunarra* numbers and a converse positive effect on wasp numbers, which was attributed to the high densities of wasp nests in the adjacent plantation forests. Quantifying the density of vespid wasp nests within plantation forests requires further study. An optimal buffer width of 300-500 m was determined, with 500 m being preferable, as wasps generally fly 400 m from the nest when foraging (Archer 2012) (Chapter 5).

These buffers have the potential to reduce wasp incursions into the *Poa* grasslands without the use of toxic baiting, which would make it easier to manage the threat in the long-term, as less labour would be required to control wasps once the buffers were established for the protection of *O. ptunarra*. A reduction in the use of poisons would also be a good outcome for the ecosystem. As buffer zones are already used to protect some threatened species in Tasmania and have previously been used to protect reserves from incursions by wasps, it is advisable that government regulators also create a policy to place buffer zones around populations of *O. ptunarra* for their protection. Such a policy would be mandatory for the forest industry and private forest companies and other relevant landowners planting and harvesting forest near *O. ptunarra* habitat.

Buffers could be put in place over time, for ease of implementation. To begin with, areas of native vegetation within the buffer zones could be rehabilitated if necessary and then retained intact. Next, younger plantations could be cleared and rehabilitated back to native vegetation. Finally, when an established plantation is due for harvest, areas that occur within the buffer zone could be rehabilitated back native vegetation, following harvest. Care should be taken to cover disturbed ground after harvest and rehabilitation works with dense weed matting or similar to prevent wasps from digging nests in these areas. Performing harvest operations and rehabilitation works during winter when the wasps are hibernating would also limit a wasp incursion.

At *Poa* grassland sites where *O. ptunarra* has become extinct, due to the predation pressure of vespid wasps or for other reasons, it would be beneficial to translocate *O. ptunarra* to the sites to replenish them, as it is unlikely that the butterflies will fly the long distances between fragmented habitat to naturally recolonise the uninhabited sites. Translocations should be performed with caution and used only as an interim tool, while other conservation actions are being developed to effectively protect the species from the threat of predatory wasps, habitat loss and degradation, and fragmentation. The *O. ptunarra* translocations in the present study were successful at one of four release sites in the highland *Poa* grasslands of north-western Tasmania, with a persistent population of butterflies being detected during monitoring surveys conducted in the following four years (Chapter 4). A successful translocation is generally classified as one where the species survives at least 3 years in the wild (Oates and Warren 1990). The *O. ptunarra* translocations in the present study had a 25% success rate, which is comparable to the 26-38% success rate for butterfly translocations performed on various species in Britain and Ireland (Oates and Warren 1990).

The fact that less than 1% of the butterflies collected from the period 2009-2012 perished during the translocations (Chapter 4) indicates that the methods used were suitable, with most butterflies and eggs being successfully caught, transported and released without mishap. The decrease in the numbers of eggs collected from captured imagoes over the study period was attributed to capturing the females for egg collection too late in the season, when they had already laid most of their eggs. In future, it would be advisable for egg collections to take place about a week before the end of March and for the vials to contain cuttings of *Poa*.

Most successful translocations occur when suitable habitat is present at the release site and there is supporting site management (Oates and Warren 1990). Consequently, it is more likely that choosing sites with the type of vegetation composition that the butterflies prefer would lead to greater chances of translocation success and of the butterflies becoming established. *O. ptunarra* were more likely to be present at *Poa* highland grasslands sites containing the species *Poa labillardierei* and *P. hiemata* and a high abundance of flowering nectar plant species (Chapter 4). This result is consistent with that of Anderson (2010) who determined that, under laboratory conditions, *O. ptunarra* showed a preference for *Poa hiemata*.

The positive relationship between flower richness and translocation success indicated that *O. ptunarra* imagoes require nectar to successfully breed and lay sufficient eggs for the population to remain viable. Thus, high flower richness is an essential attribute for future translocation sites. Previously, it was thought that *O. ptunarra* did not require nectar to survive (Anderson 2001), but the present study shows that this is not the case at the population level and that *Poa* grasslands with few flowering species rarely supported populations of *O. ptunarra*. Ongoing management of the sites should be continued to reduce invading shrubs and to keep herbaceous vegetation within the grasslands from becoming overgrown.

6.3 Further conservation management

6.3.1 Recovery planning

The overall objective of the most recent Recovery Plan for *O. ptunarra* is to achieve downlisting of the species to a lower risk category within five years based on IUCN criteria (Bell 1999). Unfortunately, this outcome has not been attained. If anything, the species has declined, with the most likely cause being vespid wasp predation.

The lack of funding for threatened species recovery in Tasmania is woeful and made starkly apparent by the fact that the latest Recovery Plan, which was a 5-year plan for the period 1998-2003, has not been updated and is now over 15 years out of date (see Bell 1999). Similarly, the listing statement for the species, which was published in 1998 as an information guide for the public, has not been updated (see Threatened Species Unit 1998). Funding of environmental programs by both state and federal governments declined by an estimated 9.7% during the period of 2013–2014 to 2016–2017 (Australian Conservation Foundation 2018). Furthermore, in 2018, federal funding for biodiversity conservation accounted for only 5 cents in every $100 of government expenditure, its lowest level in more than a decade (Australian Conservation Foundation 2018). Greater funding is required within both State and Federal governments for threatened species management for the adequate protection of *O. ptunarra* in the long-term.

Of the six recovery actions outlined in the Recovery Plan, one has been completed by the present study, with the remainder being partially completed, ongoing, or incomplete (Bell 1999). The recovery action completed by the present study is to start a new colony of *O. ptunarra* by translocation (Chapter 4). The ongoing actions are to: protect habitat at specific populations; provide advice and information to land owners and managers; and monitor habitat and population density at selected sites. These actions are still ongoing to a limited degree in conjunction with other regulatory bodies but are restricted due to the lack of resources. The revision of the taxonomy of the species has been partially completed, with a study on the morphometrics of the species published in 1997 (McQuillan and Ek 1997). However, further genetic analysis of *O. ptunarra* is required to determine if the population found in the north-west of Tasmania constitutes a distinct subspecies. The incomplete action is that the conservation status of the species has not been reviewed.

6.3.2 Reducing habitat loss

Further *O. ptunarra* habitat loss could be reduced by a combination of consultation with land managers and legislation. As many *Poa* grasslands are now protected under federal and State government legislation, it is expected that clearing of these vegetation communities will be minimised. Ongoing communication with land managers that have *O. ptunarra* on their properties to make them aware of the status and requirements of the species could also assist in protecting the habitat of *O. ptunarra*. Many landowners were previously been made aware of the occurrence of *O. ptunarra* on their properties many years ago but should be updated regularly with new information about the species. Additionally, properties may have changed hands in the interim and the new landowners may not be aware of the presence or management of *O. ptunarra*. Placing management agreements and private covenants on *O. ptunarra* populations on private land would allow for greater protection of the species in the long-term. There are not currently any government incentives available for landowners wanting to protect *O. ptunarra* habitat on their properties, but they do arise occasionally.

6.3.3 Habitat management

The *Poa* grasslands require light grazing or intermittent fire to keep the grassland at an early successional state suitable for *O. ptunarra*. If fire and grazing are absent, the tussocks often become overgrown and shrubs start to invade the grasslands as they succeed to the next vegetation type (Neyland 1993). In these instances, butterfly numbers are also usually low, as there are few inter-tussock spaces for butterflies to fly, or for flowering herbs to grow.

The recommended burning regime to manage *O. ptunarra,* of patch-burning in a mosaic style every 5-10 years in late autumn to winter, remains appropriate (Bryant and Jackson 1999). This time of year is preferable as adult butterflies are not present and larvae are likely to be sheltering deep within the tussock and are less likely to be killed. Many *O. ptunarra* populations on private land in north-western Tasmania and the Midlands are effectively managed this way by the landowners (Anderson 2001).

In the Midlands, light grazing is also used to manage *O. ptunarra* habitat from becoming overgrown. Sheep grazing is preferred over cattle grazing, as the larger cattle can pull out larger tufts of grass and sometimes the whole tussock. This can result in the death of the tussock and potentially the decline of the grassland (Anderson 2001). To maintain healthy *Poa* grasslands that will benefit *O. ptunarra* populations, a light sheep stocking rate of around 2-3 DSE (dry sheep equivalent) per hectare is recommended (Anderson 2001).

There are also many areas of *O. ptunarra* habitat that are not being effectively managed, which are becoming overgrown and may not be suitable habitat for the species in the near future if left unmanaged. These grasslands require further attention from land managers, either through patch-burning, removal of woody vegetation, or by light sheep grazing. Land managers could apply for funding to rehabilitate threatened species habitat through the NRM bodies or Landcare Tasmania, who have annual funding rounds for small conservation projects.

6.3.4 Agricultural chemicals & GM

The threat of agricultural practices such as the spraying of pesticides and herbicides, application of fertilisers, and use of genetically modified (GM) crops has not been greatly considered in the conservation management of *O. ptunarra*. Agricultural chemicals are regularly used on paddocks and crops adjacent to *O. ptunarra* colonies in the Tasmanian Midlands (Anderson 2001) and herbicides and pesticides are often used in plantation forests (Neyland and Brown 1996). Spray drift of these toxins across the grasslands has the potential to impact on *O. ptunarra* populations (Neyland and Brown 1996). Anderson (2001) suggested that these chemicals did not appear to be having an adverse effect on *O. ptunarra* in the Midlands. However, further investigation is warranted, focusing particularly on the larval stage. There is currently a moratorium on the use of GM crops in Tasmania, so these do not currently pose a risk to *O. ptunarra* (DPIPWE 2014). However, the moratorium is in the process of being reviewed and, if GM crops are permitted in the future, then their impact on nearby *O. ptunarra* populations and habitat will require close monitoring.

6.3.5 Climate change

The threat of climate change to *O. ptunarra* populations has not been considered much, nor addressed. Climatic modelling suggests that the Tasmanian highlands will get drier and warmer (Grose et al. 2010) and climatic observations show an increase in extreme dry and wet periods, and more wildfires due to an increased occurrence of dry lightning in the west of the State (Styger et al. 2018). These climatic changes are likely to alter the distribution of vegetation communities, which may impact on *O. ptunarra* habitat.

Modelling of the impacts of climate change on the closely related *Oreixenica correae* on mainland Australia suggested that its range would contract and change location (Beaumont and Hughes 2002). This suggests that *O. ptunarra* should also be monitored for potential climate induced changes, which will potentially change the landscape in which *O. ptunarra* resides. Further research and modelling are required to determine the impact of climate change on *O. ptunarra* and how to ameliorate these impacts.

Wasps are also likely to be affected by climate change. A study of the response of vespid wasps to climate change in New Zealand and the UK predicted that, in areas where there is a decline in precipitation and an increase in temperature, there will be an increase in annual wasp abundances (Lester et al. 2017). Observations in Poland show this population change is happening (Tryjanowski et al. 2010). As climate modelling predicts increasing temperatures in Tasmania, increases in vespid wasp numbers are likely, mainly in years that correspond with extreme low rainfall events in the springtime. However, it is also likely that there will be years of low wasp numbers, when extremely high rainfall events occur in the springtime, causing flooding in early-stage wasp nests, resulting in their demise.

6.3.6 Captive propagation

Captive rearing can be used as a tool to maintain threatened populations in the short-term, by temporarily increasing the size of the population, but it is not a long-term solution as declining populations often become extinct unless population growth rates change (Crone et al. 2007). Captive rearing can be a useful tool for managers to give them time to prevent the causes of the species' decline but should not be used at the expense of creating and maintaining self-sustaining populations (Crone et al. 2007). Generally, captive rearing has not been beneficial for rapidly declining or highly stochastic populations, as their numbers decrease quickly, even with supplementation (Crone et al. 2007).

A review of captive propagation studies in the USA showed that one-third of the programs had been discontinued and only 50% of programs had resulted in the release of individuals into the wild, presumably due to the complexities involved (Schultz et al. 2008).

Captive propagation could be a useful tool to supplement ailing *O. ptunarra* populations but is costly and complex. Previous small-scale rearing trials were performed in Hobart with limited success, as it proved difficult to rear the species to maturity in the laboratory, with larvae often succumbing in the first instar (Anderson 2010). Future research would be required into successfully rearing *O. ptunarra* in captivity before this method could be relied upon as a conservation approach.

6.4 Conclusion

Invasive vespid wasps are having a negative impact on threatened *O. ptunarra* butterflies through predation. *O. ptunarra* numbers are significantly less at sites where vespid wasps occur and have undergone a reduction since vespid wasps appeared in the late 1990s. *O. ptunarra* numbers are being kept at low densities but could increase again if vespid wasp numbers are sufficiently reduced.

To further reduce vespid wasp numbers to effectively protect *O. ptunarra*, the wasp control effort needs to be increased, in conjunction with other conservation measures, such as buffers. It was determined that a buffer width of 300-500 m would best protect *O. ptunarra* from incursions by vespid wasps nesting in the adjacent plantation forests. A new policy is required from government regulators to make these buffers a legislative requirement.

Translocation was determined to be an effective conservation method for restocking sites where *O. ptunarra* becomes locally extinct due to vespid wasp predation or for stochastic reasons. Translocation sites should have a mixture of *Poa labillardierei* and *Poa hiemata* and a high number of flowering plant species to ensure a high likelihood of success.

Greater government funding for threatened species management is required to enable the recovery of the species. *O. ptunarra* is but one of many species that are currently underfunded and have outdated recovery plans and listing statements, and little on-ground action to further the knowledge of the species and ensure their ongoing protection.

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Appendix A

Listing statement: ptunarra brown butterfly (*Oreixenica ptunarra*)

THREATENED SPECIES LISTING STATEMENT

Ptunarra Brown Butterfly, *Oreixencia ptunarra* Couchman 1953

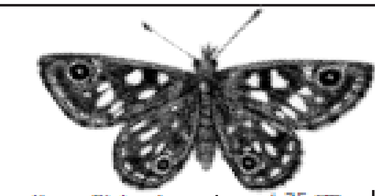
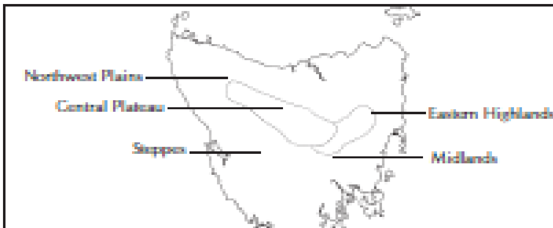


Illustration: Karen Richards



Known distribution of the Ptunarra brown butterfly

Status

Commonwealth *Endangered Species Protection Act 1992*..............Not listed
Tasmanian *Threatened Species Protection Act 1995*..............Vulnerable

Description

The Ptunarra brown butterfly is a small brown and orange butterfly belonging to the family Nymphalidae. Three sub-species of the butterfly were described by Couchman (1953). *O. p. roonina*, from the Midlands, North-west Plains and lower Steppes is the largest of the three, with a wingspan of 30-33 mm. The white background colour in the male sometimes extends as bands across the wing. *O. p. angeli* from the Eastern Highlands is intermediate in size to the other two races, with a wingspan of 27-29 mm. The background colour in the male is yellow, not white as in *O. o. roonina*. The Central Plateau form, *O. p. ptunarra*, is the smallest and darkest, with a wing span of 25-26 mm with the cream background colour in the male appearing only as small spots. The females are similar in size to the males but are distinctly different in colour. The female is light orange-yellow with faint light brown basal areas and two short bars on the front margins of the fore wings. Both wings carry wing spots as in the male (Neyland 1991).

The fully-fed larva is about 19 mm long and 4.5 mm wide and tapers sharply from head to tail. The segments are greenish grey, lightening in tone towards the head and tail. An olive brown dorsal line is bounded on either side by a narrow cream line, while a narrow median line is olive brown, a narrow subspiracular line and a spiracular band are cream in colour. The head has a few scattered black hairs.

The pupa is about 9.5 mm long and 3.5 mm wide at the level of the wing covers. It is greenish-grey flecked with black, with a pair of black spots on each body segment.

Life Cycle

The flying season lasts for two to three weeks in early autumn. During this period, eggs are laid in tussock grass and after about six weeks the larvae hatch. The larvae remain largely inactive during the winter, then during the following spring they feed on tussock grass tips. Pupation takes place deep within the tussock grass and lasts up to five weeks. In March the adults emerge, males before females, and butterflies at higher altitudes before those at lower elevations.

Distribution and Habitat

The Ptunarra brown butterfly is endemic to Tasmania and restricted to five areas of the state: the Midlands, Steppes, Northwest Plains, Eastern Highlands and the Central Plateau.

The range of the Ptunarra brown butterfly is determined by a variety of environmental factors. It is generally a montane to alpine species being restricted to sites above 400 m. It does not extend into the lowland plains of the Midlands, where it may be too warm for the butterfly and where it is too dry for its food plant to flourish. In the north-west the butterfly is limited by the availability of habitat (Neyland 1992). Habitat modelling indicates that the butterfly currently occupies its full potential range, with the possible exception of the north-east highlands.

Throughout its range the Ptunarra brown butterfly is found in areas where there is a significant cover of *Poa* tussock. Some apparently excellent sites do not carry butterflies and this may be due to the history of the site. It is possible that the species has been eradicated from the western Central Plateau by a European history of over-firing and overgrazing. The preferred habitat ranges from *Poa* tussock grassland to *Hakea microcarpa* grassy shrubland to *Eucalyptus* grassy open woodland.

Threats and Limiting Factors

The Ptunarra brown butterfly is absent from areas which have been converted to pasture. Butterfly habitat has been lost as large areas of native grassland and grassy woodland have been converted to pasture. Many populations are now found on the fringes of areas which once would have supported large colonies. (Neyland 1992). Loss or reduction and fragmentation of available habitat caused by land clearing has threatened the species (Neyland 1993).

Grazing intensity affects the population size of the Ptunarra brown butterfly but the exact relationship between grazing pressure and butterfly numbers is not fully understood. Few butterflies are found on sites which are heavily grazed but in areas where there has been little or no grazing and where the tussocks have become large and overgrown, butterfly numbers are also low (Neyland 1992). Some grazing appears to be beneficial to the butterfly as it often avoids dense grasslands.

In the Northwest Plains, large areas of *Poa* dominated grasslands and grassy woodlands, which are naturally restricted in area, have been converted to eucalypt plantations by private forest companies (Neyland 1992).

Repeated burning of remnant native grassland has caused a severe decline in population levels of the Ptunarra brown butterfly in some areas. However, too infrequent firing promotes invasion of native grassland by shrubby species, reducing the cover of *Poa* and the attractiveness of the habitat to the butterfly.

The Ptunarra brown butterfly is a weak flyer and the probability of recolonising sites, unless suitable habitat corridors exist, appears low. If small remnant populations are lost, through overgrazing, fire or clearing, then those sites may never be recolonised.

Conservation Assessment

Historical Distribution

Historically, the range of the Ptunarra brown butterfly is thought to have been widespread in *Poa* grassland, shrubland and open woodland habitats across central Tasmania. However, the butterfly has undergone a substantial reduction in area of occupancy since European settlement. In the Midlands, less than 3% of the original extent of native grasslands remains intact (Fensham & Kirkpatrick 1989) and throughout the State 40% of the original area of *Poa* grassland has been lost since 1802 (Kirkpatrick *et al.* 1995).

Area Currently Occupied

There are 150 colonies of Ptunarra brown butterfly currently known, covering an area of approximately 13,900 ha. *O. p. angeli* comprises 35 colonies over 1,200 ha, *O. p. roonina* comprises 82 colonies over 7,400 ha and *O. p. ptunarra* comprises 33 colonies over 4, 300 ha.

Population Estimate

Table 1: Population estimate of *O. ptunarra* according to bioregion (Orchard 1988) throughout its range.

Biogeographic region	1997 population (no. of sites, total area)
Midlands	91 000 + 58 000 (32, 4 100 ha)
Eastern Highlands	42 000 + 29 000 (35, 1 200 ha)
Steppes	55 000 + 35 000 (23, 1 500 ha)
Central Plateau	115 000 + 98 000 (33, 4 300 ha)
Northwest Plains	114 000 + 77 000 (27, 3 300 ha)

Reservation Status

Six known protected colonies comprising about 600 ha occur within the Central Plateau Protected Area of the World Heritage Area and 50 ha within Cradle Mountain/Lake St Clair National Park, also within the WHA.

Approximately 6% of the known range of the species is reserved and approximately 76% of the total population occurs on private land.

Approximately 2% of the total area of *Poa* grassland is reserved in Tasmania representing a total area of 1,183 ha (Kirkpatrick *et al.* 1995).

Several areas containing Ptunarra brown butterfly colonies on land owned by North Forest Products in the Northwest Plains are contained within a private reserve system and are protected from conversion to plantation.

Assessment Criteria

By the 1980's it was recognised that the Ptunarra brown butterfly was in decline. In 1988 only 33 locations were known for the butterfly and based on IUCN criteria (i.e. Wells *et al.* 1983), Prince (1988) considered *O. p. ptunarra* to be secure, while *O. o. roonina* and *O. p. angeli* were considered endangered. Following further research, Neyland (1991) considered all sub-species to be endangered.

The identification of a large number of previously unknown colonies throughout the range of the butterfly, co-operative management agreements with private landowners and increased public awareness have improved our knowledge of the status of the species in recent years.

To ensure the continued survival of the species there should be no further loss and fragmentation of known colonies.

The majority of known colonies on private land remain threatened and require sympathetic land management practices.

Recovery Program

A national recovery plan was prepared in 1991 (Neyland 1991) which is due for revision and re-submission to Environment Australia.

Recovery Program Objective
Downlisting of *O. p. angeli* to Rare within five years and stabilisation of *O. p. roonina* within five years with possible downlisting to Vulnerable within ten years.

Recovery Program Criteria
The criteria state that:

- Landowners need to co-operate to manage *O. ptunarra* habitat.
- An increase in the numbers of *O. ptunarra* at a selection of sites will be needed to be demonstrated through a monitoring program.
- Public education programs will be needed to increase awareness of the species.
- Existing colonies will be needed to be protected through sympathetic land management practices, fencing and/or reservation.

Recovery Program Evaluation
The program has met its objective with listing of *O. ptunarra* as Vulnerable on the *Threatened Species Protection Act 1995*. Downlisting of the species to a lower risk category (IUCN criteria) is sought within 10 years.

Existing Management

Private Land
North Forest Products has a private reserve system in place for the protection of several butterfly colonies in the Northwest Plains.

Some landowners in the Midlands and Central Plateau use farm management practices that are sympathetic to conservation of the butterfly. Land management agreements are being developed that consider management of the butterflies' habitat.

Commercial Forestry
Colonies affected by commercial forestry operations are identified in timber harvest plans through the Forest Practices Code (Forestry Commission 1993) and are protected from logging.

Required Actions

Habitat Management

- Develop land management agreements with private landowners to secure the butterflies' habitat.
- Monitor butterfly populations and prevent degradation of habitat.

- Develop concise guidelines for management of the butterflies' habitat.
- Increase awareness of the butterflies and their habitat and provide information on conservation of habitat to landowners.
- Retain *Poa* grasslands within the range of the Ptunarra brown butterfly.
- Promote light grazing in *Poa* grasslands and minimal use of fertilisers within the butterflies' range.

Information Needed

- Long-term monitoring of a range of sites is essential in order to measure the effects of grazing pressure and fire regimes on butterfly populations and determine relative population densities and trends over time.

Source Material

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Peter Brown, Threatened Species Unit, Parks and Wildlife Service, Tasmania.

Mark Neyland, Forestry Tasmania.

Review and Further Information

Statement Prepared: April 1998

Prepared By: Phil Bell

Review Date: Expiry of the new recovery plan or as new information is received.

Cite As: Threatened Species Unit 1998 Listing Statement Ptunarra Brown Butterfly *Oreixenica ptunarra*, Parks and Wildlife Service, Tasmania.

Further Information: Threatened Species Unit, Parks and Wildlife Service, GPO 44A Hobart Tasmania 7001.
Ph (03) 62 33 6556 fax (03) 62 33 3477

Permit: It is an offence to collect, possess or disturb this species unless under permit from the Director, PWS.